

Surgical Forum

Proceedings of The Forum Sessions
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of Surgeons San Francisco California
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Surgical Forum

VOLUME VII

Surgical Forum Committee

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Foreword

Each year an increasing number of recently completed research projects are submitted to the Forum Committee for consideration for the annual Forum on Fundamental Surgical Problems. Judging from the abstracts submitted I am convinced that they are increasing in excellence as well as in number. They represent a wide variety of basic investigations in general surgery and in the surgical specialties. From these abstracts the Committee is able to select a program of unsurpassed quality. This volume contains brief, carefully written and illustrated descriptions of the research efforts presented at the annual Forum. It is a volume that should be in the hands of all those who are interested in current progress in surgery.

These papers are representative of the best which is being accomplished by young American surgeons. They constitute a tribute to the growing interest, enthusiasm and investigative capacity of these young surgeons. They reflect the steadily increasing availability of proper research facilities, both laboratory and clinical. They were made possible by research funds from government agencies, private foundations and local institutions. This wholesome state of affairs speaks well for the future of surgery.

The members of the Forum Committee express their appreciation to Helene Coleman for her excellent work in editing the manuscripts included in Volume VII of the **SURGICAL FORUM**.

HARRIS B. SHUMACKER, JR.

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TOLERANCE OF DOGS RESUSCITATED WITH DEXTRAN TO SUBSEQUENT SURGICAL STRESS*

CLOTTREY SLANEY

Experiences in the Korean war proved beyond doubt the value of Dextran as a resuscitative agent when used in quantities of the order of 1000 cc to 1500 cc.¹⁻³ Experimentally, it has been shown that Dextran will adequately resuscitate animals which have undergone severe blood loss of 10 to 50 per cent of the blood volume.⁴ As far as can be ascertained, however, no experimental work has been done on how well animals thus resuscitated withstand subsequent major surgery. As this may have important clinical implications this research was undertaken to study the response of animals to a major operative procedure following severe blood loss corrected by Dextran replacement.

METHOD

Sixty-nine dogs were used as the experimental subjects and they were divided into 2 groups. Group 1 consisted of 19 dogs and group 2 of 50 dogs. All experiments were performed under morphine and nembutal anesthesia.

Group 1 consisted of 9 dogs which served as control animals and 10 dogs which were the experimental animals. The control animals were bled 10 to 50 cc per kg of body weight, this blood being collected in a heparinized container and later retransfused back into the animals. The period of hypotension following bleeding averaged 5 minutes or so during which the blood pressure was 20 to 40 mm Hg. When retransfusion had been completed a stress operation consisting of cholecystectomy, splenectomy and left nephrectomy was then performed. Exactly the same procedure was followed in the experimental animals except that the blood withdrawn was replaced by an equal volume of 6 per cent Dextran in 0.9 per cent saline.

Group 2 consisted of 25 control dogs and 25 Dextran dogs. The same procedure was followed as in group 1 but the stress was considerably increased by bleeding all the animals a further 15 cc per kg of body weight (blood not replaced) after the operation had been completed.

RESULTS

The group 1 experiments were designed to simulate patients who had undergone massive Dextran replacement and then had to undergo a major operative procedure without further blood loss. The mortality rate in this group was 20 per cent in the Dextran resuscitated dogs as compared with 0 per cent in the control animals.

*From the Department of Surgery, University of Illinois College of Medicine, Research and Educational Hospital, Chicago, Illinois. Supported in part by a grant from the Graduate School of the University of Illinois.

The Dextran used was supplied by Abbott Laboratories.

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Shock

TOLERANCE OF DOGS RESUSCITATED WITH DEXTRAN TO SUBSEQUENT SURGICAL STRESS*

CLOFFREY SLANEY

Experiences in the Korean war proved beyond doubt the value of Dextran as a resuscitative agent when used in quantities of the order of 1000 cc to 1500 cc.¹⁻³ Experimentally it has been shown that Dextran will adequately resuscitate animals which have undergone severe blood loss of 40 to 50 per cent of the blood volume.³⁻⁵ As far as can be ascertained however no experimental work has been done on how well animals thus resuscitated withstand subsequent major surgery. As this may have important clinical implications this research was undertaken to study the response of animals to a major operative procedure following severe blood loss corrected by Dextran replacement.

METHOD

Sixty nine dogs were used as the experimental subjects and they were divided into 2 groups. Group 1 consisted of 19 dogs and group 2 of 50 dogs.

All experiments were performed under morphine and nembutal anesthesia.

Group 1 consisted of 9 dogs which served as control animals and 10 dogs which were the experimental animals. The control animals were bled 40 to 50 cc per kg of body weight, this blood being collected in a heparinized container and later retransfused back into the animals. The period of hypotension following bleeding averaged 5 minutes or so during which the blood pressure was 20 to 40 mm Hg. When retransfusion had been completed a stress operation consisting of cholecystectomy, splenectomy and left nephrectomy was then performed. Exactly the same procedure was followed in the experimental animals except that the blood withdrawn was replaced by an equal volume of 6 per cent Dextran in 0.9 per cent saline.

Group 2 consisted of 25 control dogs and 25 Dextran dogs. The same procedure was followed as in group 1 but the stress was considerably increased by bleeding all the animals a further 15 cc per kg of body weight (blood not replaced) after the operation had been completed.

RESULTS

The group 1 experiments were designed to simulate patients who had undergone massive Dextran replacement and then had to undergo a major operative procedure without further blood loss. The mortality rate in this group was 20 per cent in the Dextran resuscitated dogs as compared with 0 per cent in the control animals.

*From the Department of Surgery, University of Illinois College of Medicine, Research and Educational Hospital, Chicago, Illinois. Supported in part by a grant from the Graduate School of the University of Illinois.

The Dextran used was supplied by Abbott Laboratories.

The group 2 experiments were designed to simulate clinical conditions where following a massive Dextran replacement a patient might have to undergo subsequent major surgery accompanied by further frank or latent blood loss. Among the control dogs in this group the mortality rate was 4 per cent, whereas in the Dextran dogs it was 72 per cent. The experimental animals in this group were unable to tolerate the final bleeding procedure whereas the control animals could do so. In the Dextran dogs the immediate postoperative blood pressure was satisfactory but fell precipitously following the final hemorrhage and complete circulatory collapse ensued. The tolerance of these animals to hypotension following the initial hemorrhage was also reduced as compared with that of the controls.

The results of this study were expressed as mortality rates in each group and were as follows:

Group 1 Bleeding + Stress Operation		Mortality Rate
7 Control dogs	Blood replaced	0%
10 Dextran dogs	Blood replaced with Dextran	20%
Group 2 Bleeding + Stress Operation + Additional Bleeding		Mortality Rate
25 Control dogs	Blood replaced	4%
25 Dextran dogs	Blood replaced with Dextran	72%

DISCUSSION

After operation all animals that survived recovered consciousness within 8 hours and many achieved this state within 2 hours. With 2 exceptions all animals that did not recover within 8 hours were already dead or were in established progressive peripheral circulatory failure and died shortly afterwards. All the non survivors died within 16 hours of the operation. In the main the Dextran dogs took longer to return to consciousness than the dogs which received blood. Constant low grade capillary oozing from the abdominal incision was the usual finding in the vast majority of the dogs which had received Dextran and there can be no doubt that these animals had an impairment of the blood coagulation mechanism. Usually the oozing from the incision had ceased by the time the wound was closed but this was not invariably the case.

The autopsies on the Dextran dogs which died showed essentially similar findings. There was a variable quantity of blood stained fluid in the peritoneal cavity the quantity roughly corresponded to that found in control animals sacrificed at a similar time postoperatively. The stomach, small bowel and colon were ashen white, virtually bloodless and the musculature strongly contracted but in no case examined was there any evidence of hemorrhage into the bowel lumen or necrosis of the mucosa. The liver was extremely pale and shrunken in size. The remaining kidney was examined in approximately one third of the cases and apart from its extreme pallor appeared microscopically normal. No evidence of infarction was seen as reported by Pirani and his associates.³ The thoracic viscera all appeared normal apart from pallor and the usual terminal congestion of the lower lobe of the dependent lung.

CONCLUSIONS

- 1 The tolerance of dogs resuscitated with Dextran to subsequent surgical stress is lower than that of dogs resuscitated with blood
- 2 Animals resuscitated with Dextran and undergoing subsequent surgery will not tolerate further blood loss and should this occur, additional replacement should be with blood and not with more Dextran
- 3 Animals resuscitated with Dextran have a much reduced tolerance to hypotension as compared with animals resuscitated with blood
- 4 There is a definite hemostatic defect after infusions of large volumes of Dextran. Despite investigations by numerous workers the nature of the defect remains obscure and awaits further elucidation
- 5 For hemorrhage of moderate degree Dextran is very effective as a blood substitute

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A SIMPLIFIED METHOD FOR BLOOD VOLUME DETERMINATIONS USING RADIOACTIVE ISOTOPES*

WILLIAM A. SPENCER, J. RICHARD THISTLETHWAITE AND
SOLOMON N. ALBERT

Our interest in the physiological variations in blood volume experienced during anesthesia and surgery prompted our efforts toward the development of a simple technique for liquid phase counting adaptable to both the laboratory and the operating room.

A plastic coil was developed for use with a well type scintillation counter. The coil is prepared from Clay Adams PE 260 polyethylene tubing internal diameter .07 inches. The tubing is wound around a glass rod and inserted into the well counter. The introduction of the coil has efficiently replaced the standard aliquot measuring techniques.

METHOD

CR¹ tagged red blood cells may be used with the coil. The patient's own blood is tagged or a stock preparation of O Rh negative tagged cells is used.

From the Departments of Anesthesiology and Surgery, District of Columbia General Hospital and George Washington University Hospital, Washington, D.C. From the Anesthesia and Surgery Research Laboratory, District of Columbia General Hospital. Supported in part by the U.S. Atomic Energy Commission, Contract AT (30-1) 1820.

The stock cells are prepared by adding 1 mc of sodium chromate (R) to 500 ml of whole blood in 150 ml A C D solution. Ten to 20 ml of cell pack is removed from the stock solution and washed twice before being resuspended in normal saline to make a total volume of 25 to 30 ml.

When determining blood volume the cell suspension of standard is counted in the coil at a pre set time. Twenty milliliters of this suspension containing approximately 15 to 20 microcuries is administered to the patient. Allowing 15 minutes for adequate mixing a sample is withdrawn from the patient and counted through the coil at the same pre set time used for the standard. The patient's blood volume is then calculated by the following formula:

$$\frac{\text{counts of standard} \times \# \text{ ml administered}}{\text{counts from patient} - \text{background}} = \frac{\text{total blood}}{\text{volume}}$$

Between each determination of standard and dilute sample the coil is washed with 10 ml of normal saline solution and the background count is rechecked.

Since there is no adherence of activity to the coil repeated determinations are possible. This merely requires determining the patient's background before the administration of subsequent doses of standard. The formula is then modified as follows:

$$\frac{\text{counts of standard} \times \# \text{ ml administered}}{\text{counts of patient} - \text{patient background}} = \frac{\text{total blood}}{\text{volume}}$$

Serial determinations may be carried out with as many doses of the standard as required being given as long as the patient's retained activity does not exceed 250 microcuries.

The technique is the same for RISA with the exception that a 1:150 or 1:200 dilution of the standard RISA solution is prepared in plasma or whole blood. This was found to be necessary as the concentrated RISA solution adheres to the coil raising the background counts in spite of repeated washings.

RESULTS

Comparing this method for blood volume determinations with the standard aliquot methods *in vitro* we have found the volume determinations through the coils to be more accurate. A comparison between diluted and undiluted standard is presented as an example. (See Table 1.) For more complete statistical data reference is made to the original report of this technique.¹

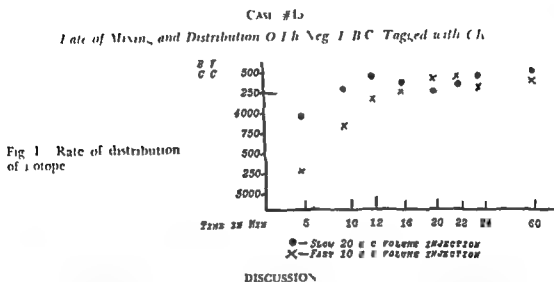
Table 1 Volume Determinations

Comparison between diluted and undiluted standards calculated from total activity

Measured vol in ml	Undiluted std		Diluted std	
	Coil	Aliquot	Coil	Aliquot
3530	3501	3385	3510	3478
3550	3546	3448	3553	3506
3570	3549	3416	3558	3543

There is no reduction in the accuracy of the volume determinations when the coil is subjected to variations in temperature from 15 to 10°C or to pressure changes up to 150 mm Hg.

The administration of 20 ml volumes of cell suspension brings about a more even distribution in a shorter period of time than does the administration of the equivalent isotope dosage in 10 ml volume or less (See Fig 1)



With the introduction of the coil we feel the technique of blood volume determinations has been greatly simplified. Utilizing the constant volume obtained with the nonvariable coil and a preset time for counting all samples has eliminated several mathematical steps.

Further simplification is manifested by a marked reduction in the technical errors encountered with the previously described methods, since our technique requires a minimum of glassware and eliminates transferring the activated sample from test tube to test tube by means of a pipette. The samples may be drawn directly into the coil from a catheter or needle in the femoral vein (see Fig 2) or may be introduced directly from the syringe used to remove the blood sample through the coil without detoured means.

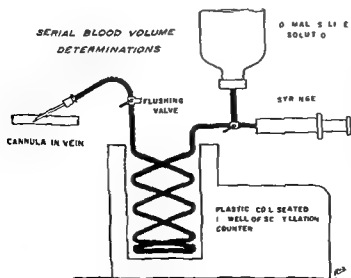


Fig 2 Diagrammatic representation of plastic coil being used with well counter for blood volume determinations

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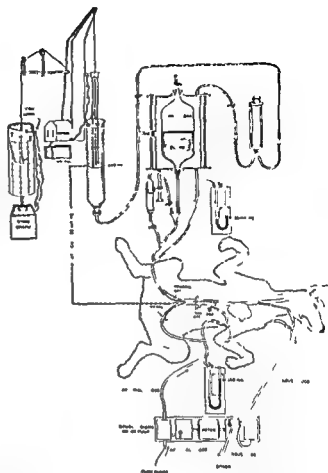
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Fig 1 The experimental set up. The dog bleeds into a limp plastic bag enclosed in a water filled tank. The blood displaces an equal amount of water into an open cylinder. Sensing electrodes connected to an electronic control maintain the water surface always at 47.4 cm above the aorta of the dog regardless of the bleeding volume by means of a reversible motor which can raise or lower the cylinder. Changes in bleeding volume are recorded on a moving kymograph by means of a pulley system connected to the cylinder. By adjustment of clamps at the end of the experiment water is pumped back into the tank with the syringe. This forces blood back into the dog through the filter.



donor dog was rendered with polyvinyl catheters in the aorta and inferior vena cava inserted via the left femoral artery and vein. A third polyvinyl catheter was placed in the right femoral artery of the donor dog to measure blood pressure. All cannulations in both experimental and donor dogs were done using local piperocaine anesthesia. Two hours following sedation the experimental dog was heparinized using 2 mg/kg intravenously and hemorrhaged to 35 mm Hg mean arterial pressure utilizing a new automatic leveling device and blood reservoir (Fig 1). Following attainment of maximal bleeding volume at 35 mm Hg ($1.15 \pm .48$ hr in 60 previous hemorrhage experiments) the superior mesenteric artery of the shocked dog was isolated through a left flank retroperitoneal approach. The artery was clamped off from the aorta and cannulated. The profound shock plus small amounts of local piperocaine anesthesia was ample for this brief procedure. Following cannulation controlled cross perfusion of the artery (double Sigmamotor pump) was immediately started at a rate of 8 to 10 ml/kg/min utilizing arterial blood from the donor (See Fig 1). Equal amounts of venous blood were returned from the perfused dog to the normotensive donor dog also heparinized 2 mg/kg. The shocked dogs mean superior mesenteric arterial pressure was maintained at about 110 mm Hg with this flow. With the use of the automatic leveling device the perfused dogs mean systemic arterial pressure was maintained at 35 mm Hg throughout the period of perfusion. When the perfused dog had been in shock $1\frac{1}{2}$ hours the perfusion was stopped, the cannula removed from the superior mesenteric artery and the artery quickly repaired with 6/0 silk. The flank wound was tightly closed.

urements This makes the technique applicable to the operating room for serial blood volume determinations during surgery yet within the realm of those having little technical knowledge regarding isotopes

At the present time William Swan M.S. working in our laboratory is assembling a dilution meter computer which will give direct blood volume readings

SUMMARY

1 A plastic coil simplifying the technique of liquid phase counting is presented

2 Both CR⁵¹ labeled red cells and radioactive iodinated human serum albumen may be used with the coil

3 Errors of manipulation and transfer are curtailed contamination is reduced and the method is adaptable for serial blood volume determinations in the operating room

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THE PREVENTION OF IRREVERSIBLE HEMORRHAGIC SHOCK IN DOGS BY CONTROLLED CROSS PERFUSION OF THE SUPERIOR MESENTERIC ARTERY*

RICHARD C LILLEHEI

The primary lesions of irreversible hemorrhagic shock in the dog are severe mucosal congestion and necrosis in the small¹ and large bowel² During prolonged hemorrhagic shock the superior mesenteric venous flow reflecting superior mesenteric arterial flow drops to less than 25 per cent of normal In order to study the effect on irreversibility of preventing these pathological changes a procedure was developed to maintain the superior mesenteric arterial flow of unanesthetized shocked dogs at normal levels

METHOD

All experiments were done in an air conditioned room and utilized unanesthetized mongrel dogs sedated with morphine sulfate 2 mg/kg intramuscularly Prior to hemorrhage polyvinyl catheters were placed in the superior and inferior vena cavae of the dog to be hemorrhaged *via* the external jugular and femoral veins Another polyvinyl catheter was placed in the femoral artery for bleed out and reinfusion An unanesthetized sedated

*From Department of Surgical Physiology Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington DC

Table 1 Hemorrhagic Shock at 55 mm/Hg Mean Arterial Blood Pressure*

TYPE OF FISTULA	NO. DOGS	NO. OF SURV. DOGS	BODY WEIGHT KG	MANIPULATED VOLUME		BLOOD VOLUME †	FISTULA START IN		FIBER NO. /MIN	HOURS IN SHOCK	HOURS TO DEATH BY SHOCK
				WHICH	DOGS		DOGS AFTER HEMORRHAGE				
Superior mesenteric arterial	30	7	107±33	60 ± 91	586±76	118±18	881±22	190±21			
Superior mesenteric arterial-fick arterial dogs	10	0	161±36	500±19	515±39	101±28	952±23	50 ± 20			639±59
None	15	1	200±51	573±55	535±16			110±10			703±61
Arteric	10	2	175±21	578±61	573±56	132±19	109 ± 21	161±20			586±31
Inferior venous	10	2	201±19	601±93	560±15	135±07	90 ± 02	191±20			

Mean values with standard deviations

† Blood volumes were not corrected for plasma trapping in the hemostatic tube or for body hematocrit

and the dog was reinfused with all shed blood in the reservoir about 5 hours following induction of hemorrhage

Control dogs similarly sedated and heparinized were hemorrhaged to 35 mm Hg and the superior mesenteric artery exposed but not cannulated. One group of control dogs received no perfusion, one group received aortic perfusion via the femoral artery and a third group inferior vena caval perfusion via the femoral vein during the shock. All control perfusions utilized the same rate of flow per kg of body weight as was used in superior mesenteric arterial perfusions.

To rule out the beneficial effect on the liver of increased portal blood flow in superior mesenteric arterial perfusions, chronic Eck fistula dogs were similarly shocked and received superior mesenteric arterial perfusion as described above. The portacaval shunts were of the end to side type.

Blood volumes were measured on all dogs before and after the experiment with a micromethod using T 1824.³ Clean surgical technique was utilized in all experiments. All surfaces exposed to blood were of non-wettable plastic and were soaked in benzalkonium chloride for 24 hours before use.

RESULTS

Table I briefly summarizes the results. Dogs receiving aortic or vena caval perfusion or no perfusion were in shock for somewhat less than 5 hours because they generally showed signs of excessive uptake and/or cardio-respiratory failure necessitating reinfusion before the prescribed period was completed. Dogs were not considered survivors unless they were alive at the end of 72 hours following the experiment. Dogs dying following the hemorrhagic shock manifested the usual signs of irreversibility. They had a recovery of blood pressure to normal levels followed by gradual decline with the onset of copious bloody diarrhea. At autopsy these dogs exhibited severe mucosal congestion and necrosis of the small and large bowel along with marked liver congestion. Surviving superior mesenteric arterial perfused dogs sacrificed at varying periods following the experiment showed normal bowels. The liver of these dogs, however, showed the same degree of congestion both grossly and microscopically as was seen in controls dying of irreversible hemorrhagic shock.

Because of the accuracy of the Sigmamotor pump it was not necessary to give the donor dogs any extra blood during the procedure. Moreover, blood volume studies done before and after experiments showed no significant blood volume changes in perfused dogs. There was no donor mortality due to irreversible shock after any of these experiments.

DISCUSSION

There are several reports of prevention of irreversibility by perfusion of the liver of dogs during hemorrhagic shock.⁴⁻⁶ All such perfusion experiments have utilized anesthetized dogs with the exception of those of Frank *et al*⁴ (1946). Moreover, these investigators have started perfusion almost coincident with hemorrhage, a fact which markedly alters the maximal bleeding volume. They have also used a considerably shorter period of shock than was used in the present studies. These factors make the comparison of liver perfusion with bowel perfusion via the superior mesenteric artery very difficult. However, Edwards *et al*⁵ (1954) showed that occlusion

Table 1 Hemorrhagic Shock at 55 mm Hg Mean Arterial Blood Pressure*

TYPE OF LIGATION	NO. DOGS	NO. OF SURV. DOGS	BODY WEIGHT KG	MAXIMAL BLEEDING VOLUME†		CIRCULATION		HOURS IN SHOCK	HOURS TO DEATH AFTER SHOCK
				BODY WEIGHT	% BLOOD VOLUME†	HOURS AFTER HEMORRHAGE	ML /KG /MIN		
Superior mesenteric arterial	30	27	19.7±3.3	6.0±0.4	38.6±7.6	1.19±.34	8.84±2.2	1.00±.23	
Superior mesenteric arterial-ileal fistula dogs	10	9	16.1±4.6	5.00±.79	51.1±3.9	1.01±.28	9.42±2.4	1.0±.20	6.50±3.9
None	10	1	20.0±5.1	5.75±.33	33.3±4.6			1.10±.10	7.05±6.1
Aortic	10	2	17.5±2.4	5.78±.61	37.3±3.6	1.12±.19	10.9±2.1	4.61±.20	
Inferior venal caval	10	2	20.4±4.9	6.04±.93	56.0±4.5	1.13±.07	9.0±.02	1.91±.29	1.46±3.1

*Mean values with standard deviations.

†Blood volumes were not corrected for plasma trapping in the hematocrit tube or for body hematocrit.

of the aorta for 2 hours above the superior mesenteric artery but below the celiac axis resulted in a higher mortality in dogs than occlusion just below the superior mesenteric artery with simultaneous hepatic artery occlusion. Parkins *et al*⁹ (1955) reported that differential intestinal cooling during a 2 hour occlusion of the thoracic aorta prevented death of dogs, whereas differential cooling of the liver did not.

Heretofore the congestion seen in the bowel of dogs dying of irreversible hemorrhagic shock has generally been attributed to hepatic vasoconstriction with resultant portal congestion. Yet Frank *et al*¹⁰ (1951) showed that chronic Eck fistula dogs were no more resistant to irreversible hemorrhagic shock than normal dogs. These experiments on Eck fistula dogs were duplicated by the author. At autopsy however, contrary to Frank *et al*¹⁰ almost the same degree of mucosal congestion and necrosis of the bowel was seen in these dogs as was seen in normal dogs dying of irreversible hemorrhagic shock. The marked mucosal necrosis present as well as the congestion has not been fully appreciated by many investigators. This necrosis apparently is the result of critical ischemia occurring during the shock period possibly enhanced by the rich bacterial flora present. Superior mesenteric arterial perfusion during hemorrhagic shock prevents these changes and prevents irreversibility from occurring. Moreover the survival of chronic Eck fistula dogs similarly perfused rules out increased portal flow to the liver as the basis for survival.

If bowel necrosis is the cause of irreversibility following prolonged hemorrhagic shock then there is a close resemblance between this condition and experimental bowel obstruction in dogs. Indeed the same abnormal hemoglobin pigment found by Nemir *et al*¹¹ (1949) in the peritoneal fluid and plasma of dogs dying of strangulation obstruction has been found by the author in the plasma of dogs dying of irreversible hemorrhagic shock. It is not present in the plasma of those dogs perfused through the superior mesenteric artery during the hemorrhagic shock.

SUMMARY

Irreversibility has been prevented in 90 per cent of a group of 30 dogs by perfusing the bowel *via* the superior mesenteric artery during hemorrhagic shock. Results were identical in a group of 10 chronic Eck fistula dogs. Bowel pathology is strongly suggested as a cause of irreversibility following prolonged hemorrhagic shock.

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A STUDY OF MITOCHONDRIAL CHANGES IN HEMORRHAGIC SHOCK*

J G STRAWITZ AND H HIFT

The advent of transfusions of unlimited quantities of blood ushered in a great advance in the prevention of death due to hemorrhage. However, it has become apparent in recent wars and in civilian practice that there is still a significant number of patients suffering from prolonged periods of hemorrhagic hypotension who are refractory to blood transfusion therapy. These patients show the signs and symptoms of the condition known as irreversible hemorrhagic shock.

It is the purpose of this study to describe some of the intracellular morphological and biochemical changes which appear to accompany irreversible hemorrhagic shock. Several studies¹⁻³ involving tissue analyses reported data which suggested that the energy metabolism of the tissues might be a site of injury. This focused our attention on the mitochondria since it had been shown in recent years that these subcellular particles are responsible for the oxidative and energy converting functions of all living cells.

METHOD

Hemorrhagic shock was produced in dogs by a modification of the Wiggers technique. The methods used in preparing formalin fixed heart mitochondria and examining them under phase contrast or isolating them fresh in sucrose suspension and determining their enzymatic activities have been described elsewhere.³ The formation of crescents was observed in mitochondrial suspensions which were diluted in sucrose solutions of suitable compositions, allowed to stand at room temperature for varying periods of time, fixed with neutral 10 per cent formaldehyde and counted under oil immersion in a Spencer phase contrast microscope. For spectrophotometric analysis the mitochondrial suspensions were diluted 1:5 with water, incubated at 37°C. overnight and centrifuged. The clear supernatants were examined in the Beckman DU spectrophotometer.

*From the Department of Surgery and Cardiovascular Research Laboratory, University of Wisconsin Medical School, Madison. This investigation was supported by the Wisconsin Heart Association, by the Department of the Army, Office of the Surgeon General, and by the National Heart Institute of the National Institutes of Health, U.S. Public Health Service.

With the technical assistance of Hackchan Rhee.

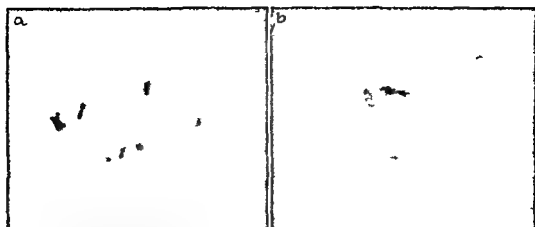


Fig 1 Heart mitochondria from normal and shocked dogs prepared in formaldehyde and examined under phase contrast

a — normal b — shock

Magnification app 2300 \times

RESULTS

In Situ Morphology Figure 1 shows free floating mitochondria which were separated from the myofibrils by blenderization in formaldehyde. In the undisturbed tissue long chains of mitochondria are arranged longitudinally on all sides of the myofibrils. The free floating normal particles tend to be square to spindle shaped rods with distinct boundaries and dense to moderately translucent centers. The particles observed in preparations from shocked dog hearts are swollen irregular in shape with indistinct boundaries and translucent centers. Blenderates from brain, liver and kidney also show abnormal mitochondria.

Behavior in Sucrose Suspension: HARMAN⁴ made the observation that mammalian mitochondria from various sources when allowed to stand in sucrose suspensions underwent a series of morphological alterations: the normal rods rounded off into solid spheres which swelled and ballooned and finally formed large signet ring shaped forms which he called "crescents" (Fig 2). He found that these transformations occurred more readily under

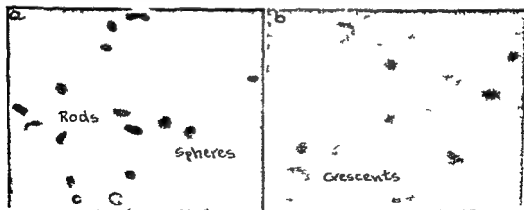


Fig 2 Heart mitochondria from a normal dog isolated by differential centrifugation suspended in 0.5 M sucrose and examined under phase contrast

a — fresh

b — after standing at room temperature

Magnification app 2300 \times

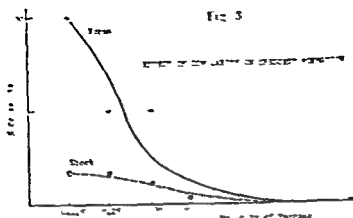


Fig 3 shows the percentage of mitochondria forming crescents in sucrose solutions of low molarity at temperatures above freezing and at neutral pH. The yields increased with time. In every instance the mitochondria obtained from the paired shocked animal underwent this transformation much less readily and the crescents which formed tended to be smaller and more compact than in the normal (Fig 3).

Fig 4

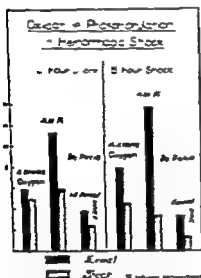


Fig 5 shows the percentage of mitochondria forming crescents in sucrose solutions of low molarity at temperatures above freezing and at neutral pH. The yields increased with time. In every instance the mitochondria obtained from the paired shocked animal underwent this transformation much less readily and the crescents which formed tended to be smaller and more compact than in the normal (Fig 3).

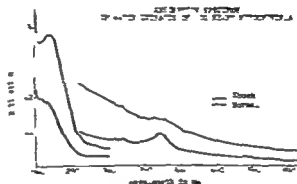


Fig 6 shows the percentage of mitochondria forming crescents in sucrose solutions of low molarity at temperatures above freezing and at neutral pH. The yields increased with time. In every instance the mitochondria obtained from the paired shocked animal underwent this transformation much less readily and the crescents which formed tended to be smaller and more compact than in the normal (Fig 3).

such environmental conditions as low tonicity and elevated temperatures. In our hands, normal dog heart mitochondria yielded large numbers of crescents in sucrose solutions of low molarity at temperatures above freezing and at neutral pH. The yields increased with time. In every instance the mitochondria obtained from the paired shocked animal underwent this transformation much less readily and the crescents which formed tended to be smaller and more compact than in the normal (Fig 3).

The water extracts derived from the mitochondria of shocked dogs generally contained more protein than similar normal preparations and had higher extinctions, especially in the flavin and UV regions (purine and pyrimidine-containing material) (Fig 4).

As described elsewhere, the oxidation of Krebs cycle intermediates and of fatty acids was essentially unimpaired even after 5 hours of shock. No requirement for coenzymes could be demonstrated. However, in the presence of the hexokinase system, oxygen uptakes and phosphate esterification were often decreased (Fig 5).

DISCUSSION

The results thus far indicate that heart mitochondria are structurally altered in irreversible hemorrhagic shock resulting in changes in size and distortion of shape. Simultaneously their oxidative phosphorylation mechanism appears to be affected. They seem to be less responsive to environmental conditions since they form crescents less readily than the normal. At the same time they apparently are more water soluble, i.e. they either contain more water soluble material or their internal substance has been altered so as to render it more soluble. At this point it is difficult to formulate a hypothesis which would account for these abnormal behavior patterns although one may be inclined to look upon them as expressions of one specific intra-mitochondrial defect which is caused by prolonged hemorrhagic hypotension.

SUMMARY

Heart mitochondria from dogs subjected to irreversible hemorrhagic shock differed in size and shape from those of normal dogs. They tended to show lowered P/O ratios in Warburg experiments, formed crescents less readily under a variety of environmental conditions and yielded water extracts which contained higher concentrations of protein, flavins and purine and pyrimidine containing material than did similar preparations from normal dogs.

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THE RELATIONSHIP OF BLOOD VOLUME TO EXCHANGEABLE POTASSIUM: AN INQUIRY INTO THE CONCEPT OF CHRONIC SHOCK *

H. VICTOR PARKER, JAMES H. McMURREY, AND FRANCIS D. MOORE

The nature and significance of the biochemical changes characteristic of cachexia are being elucidated by studies of body composition.¹ These studies show that in the absence of severe chronic hemorrhage patients suffering from starvation cachexia quite characteristically have a slightly enlarged blood volume expressed in terms of observed weight and due largely to an expansion of the plasma fraction.

*From the Department of Surgery and Laboratories for Surgical Research of the Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts. This work was sponsored by the Subcommittee on Metabolism in Trauma, Advisory Committee on Metabolism, Office of the Surgeon General, Department of the Army and supported in part by the Atomic Energy Commission.

Various therapeutic measures designed as supportive in these states are essentially empirical or based on concepts not in accord with the characteristic compositional defects as they are understood today. There is widespread acceptance of the principle of massive transfusion of whole blood in these patients preoperatively under a concept of chronic shock relating to a markedly reduced blood volume.² Clark *et al* have described blood volumes (dye hematocrit method) in patients with cachexia of a large variety of types with correction of deficits by administration of 1500 ml to 4000 ml of whole blood. In addition, there is some adherence to the suggestion of giving voluminous quantities of plasma to effect a favorable protein state.³ Volumes suggested are in the range of 2000 to 7000 ml in the period preceding surgery.

This paper attempts to define more closely this compositional disturbance relating to the vascular components and to assess its significance in chronic illness.

SELECTION OF CASES

The observations are associated with a larger study of total body composition by simultaneous dilutional techniques first outlined at this Forum in 1955.^{1,4} One hundred and twenty five such studies have now been carried out.

As the majority of our cases have been chronically ill, some aspect of depletion is usually present. However, this orientation considers a group in which cachexia secondary to a disease state has been the prominent clinical picture without distortion by an associated condition such as heart failure, renal disease or ascites. Twenty five such studies are available for selection. Factors such as duration of weight loss over one year, the presence of associated bleeding, of recently administered transfusion, of indirectly related electrolyte imbalance and the administration of cortisone further exclude the use of certain cases.

The findings in 8 males and 5 females suffering cachexia as determined by history of weight loss and clinical appearance are presented. The group encompasses a variety of disease entities and includes such surgical conditions as ulcer, carcinoma, regional ileitis, ulcerative colitis and sepsis. The average weight loss was 12.1 kg, or 19 per cent of body weight with a range of 3.1 to 19.2 kg, or from 5 to 34 per cent of body weight. None of these patients had detectable bleeding of a degree to warrant their exclusion, none had recent transfusion, none were edematous, all had histories under one year's duration and there were no other apparent complicating factors.

RESULTS

Normal values are based on a series of 20 total compositions carried out in these laboratories and on values in the literature. Data selected from the measured and derived values in each composition study for this analysis are plasma volume, red cell volume, total blood volume, total exchangeable potassium and intracellular water expressed in terms of per cent of normal.

The mean large vessel hematocrit was 87 per cent of normal with a range of 75 to 100 per cent. The mean whole body hematocrit was 76 per cent of normal with a range of 53 to 86 per cent.

The distribution of the individual values of the blood volume in relation to the exchangeable potassium is presented in Figure 1

THE RELATIONSHIP OF THE INTRAVASCULAR COMPONENTS TO K IN DEPLETION

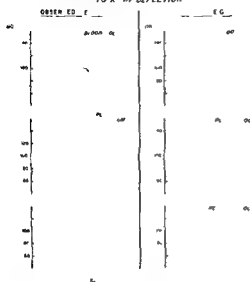


Fig. 1 The distribution of the individual values of the blood volume in relation to exchangeable potassium. The shaded areas represent the exchangeable potassium values against which the plotted individual values for blood volume, plasma volume and red cell volume are to be compared. It will be noted that the red cell mass is reduced proportionately to the K, but that plasma volume (and to a lesser extent blood volume) is significantly higher.

Total blood volumes scatter in the range above 100 per cent of normal in terms of observed weight and although slightly below the normal range for pre illness weight in neither instance are as low percentage wise as the exchangeable potassium data. Plasma volumes show a wide scatter above normal for observed weight but distribute themselves in a normal pattern for the patient's normal weight. Again they do not coincide with the range of exchangeable potassium, the lowering of the latter being extensive (as a result of tissue wasting) while the intravascular volumes are significantly higher. The red cell volumes distribute in the range of the exchangeable potassium values in both instances.

The relation of the arithmetic mean and standard deviation of the entities together with the values for intracellular water is portrayed in Figure 2.

THE RELATIONSHIP OF THE INTRAVASCULAR COMPONENTS TO K IN DEPLETION

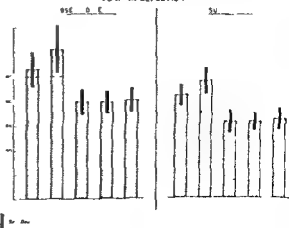


Fig. 2 The relation of the arithmetic means and standard deviations of the blood volume, plasma volume, red cell volume, exchangeable potassium and intracellular water. Again as in Fig. 1 the RCV is proportional to K and ICW whereas PV is disproportionately high.

This summarizes the changes in more graphic form. The mean plasma volume is 122 per cent of normal with a range of 96 to 162 per cent of normal for observed weight, 97 per cent of normal with a range of 89 to 113 per cent for usual weight. The mean total blood volume is 106 per cent of normal for the observed weight with a range of 83 to 130 per cent and 85 per cent of normal with a range of 76 to 99 per cent when expressed in terms of usual weight. The red cell volume in the case of observed weight has a mean value of 79 per cent with a range of 63 to 97 per cent of normal; the exchangeable potassium is 79 per cent with a range of 67 to 93 per cent; the intracellular water 81 per cent with a range of 67 to 96 per cent.

For usual weight the mean red cell volume is 63 per cent of normal with a range of 44 to 77 per cent; the mean exchangeable potassium value is 63 per cent of normal with a range of 49 to 73 per cent; the intracellular water 63 per cent of normal with a range of 48 to 76 per cent.

DISCUSSION

Total exchangeable potassium representing approximately 95 per cent of the total body content of potassium is directly proportional to the lean body mass. As such its reduction represents a significant index of wasting in the depleted patient. This premise relating to cell wasting is substantiated by the correlation found in reduction of the independently obtained value for intracellular water (total body water minus corrected bromide volume). This correlation extends to the intravascular cellular equivalent—the red cell volume. On the other hand simultaneously measured plasma volume reveals a completely independent expansion of this extracellular phase resulting in a blood volume somewhat above normal for the observed weight.

Expression of these indices in terms of weight in usual health shows an extremely poor correlation between reduction in total blood volume and exchangeable potassium and in addition gives values for plasma volume in the range of normal. The three intracellular entities show parallel defects.

CONCLUSIONS

The selection of patients as outlined in the initial section of the paper is emphasized. If chronic hemorrhage plays a role in the wasting disease significant blood volume reductions may be observed. Similarly long standing depletion and the presence of edema secondary to extremely severe depletion will influence results.

In conclusion in this selected group of chronically debilitated patients we have been unable to confirm the presence of a described syndrome characterized by consistently and often drastically reduced whole blood volumes and thus correctable by whole blood transfusion. Similarly on the basis of expanded plasma volume and expanded extracellular fluid space (these are relative expansions only) the use of large quantities of plasma in these patients should be approached with caution.

There is little clinical doubt concerning the need for accurate intravascular replacement in the preoperative period in patients with hemorrhage, acute or chronic. We are not concerned with the problem here. However in discriminate preoperative administration of whole blood based on the picture of chronic cachexia alone will inevitably result in serious overloading of the circulation in many patients. The use of separated cells would seem war-

ranted. The approach to infusion therapy requires increasing assessment in terms of selected components.⁵

These conclusions direct attention to other fields of interest: the relation of the serum albumins to the tissue proteins and closer definition of need for intravenous albumin therapy; the role of whole body hematocrits; osmolarity effects and other influences in interpretation of the hematocrit and other common clinical concentration values. (The suggestion that they are invalid because of reduced plasma volume does not seem warranted.) In addition, the significance of the parallel reduction of red cell mass with lean tissue protein should be noted in assessing "priority" concepts.

SUMMARY

1. Selected measurements from the body composition studies of 13 patients suffering from uncomplicated depletion are presented.

2. The trend of intravascular change in depletion is presented as a reduction of red cell volume paralleling the reduction in lean tissue mass; the indices for the latter being exchangeable potassium and intracellular water. The absolute plasma volume alters little and thus there is partial preservation of the total blood volume.

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CIRCULATORY RESPONSES TO INTRA ABDOMINAL MANIPULATION DURING SURGERY*

A. G. ROCCO AND L. D. VANDAM

Pronounced changes in the circulation have long been observed with intra abdominal manipulation during surgery.^{1, 2, 3} In a 1 year period at our hospital 92 or 17.5 per cent of 526 patients undergoing laparotomy exhibited noticeable falls in blood pressure. Changes in the pulse rate were less obvious but bradycardia was not an unusual finding. The nature of these circulatory responses has been studied further in 68 patients undergoing ab-

* Peter Bent Brigham Hospital, Boston, Massachusetts

dominal operations of whom 38 were women and 30 men. All categories of physical status were represented. The anesthetics given and the types of operations performed may be seen in Table 1. The anesthetics were given intratracheally in most cases by anesthesiologists of varying experience and ether was the usual anesthetic agent. Most of the cases were in the upper abdomen with cholecystectomy predominating.

Table 1 Anesthetics Given and Operations Performed During Study of Abdominal Reflexes

ANESTHETICS	NUMBER
Nitrous Oxide-Ether and Combinations with Other Agents	31
Cyclopropane and Combinations with Other Agents	15
Spinal Anesthesia and Other Conduction Anesthetics	13
Nitrous Oxide-Pentothal and Curare	4
General Anesthesia and Hypothermia	2
	—
TOTAL	65
OPERATIONS	NUMBER
Gall Bladder and Common Duct	31
Gastrectomy	3
Other Upper Abdominal Operations	6
Abdominal Vascular Surgery	6
Lower Abdominal (Gynecologic and Bowel)	22
	—
TOTAL	68

METHOD

In all patients continuous observation of the brachial arterial blood pressure was made by means of a Satham P23D strain gauge attached to an 18 gauge intra arterial needle and recorded on a direct writing Sanborn twin viso electronic recorder. Mean arterial blood pressures were obtained electronically in most cases and in a few by planimetric integration under the arterial curve. Electrocardiograms were taken in 50 patients. Since the possibility existed that changes in the circulation might be secondary to alterations in intrathoracic pressure respiratory activity also was followed by recording esophageal pressure. A plastic catheter such as is ordinarily used for nasal administration of oxygen was passed into the esophagus connected to a Satham P23D strain gauge and the pressures recorded alternately with the electrocardiogram in 12 cases.

Another explanation for the development of arterial hypotension lay in the possibility of increasing depth of anesthesia as surgical exploration was carried out. At this time a maximum depth was needed. To make certain of a constancy of anesthetic level electroencephalographic monitoring of the level of anesthesia was carried out in 25 cases with fronto occipital leads on a Grass electroencephalograph.

The aforementioned recordings were started early in the course of anesthesia. Correlations were made between changes in the records and activity in the surgical field. When the peritoneal cavity was entered a deliberate series of maneuvers was carried out to ascertain the effective stimuli responsible for development of the expected circulatory changes. Accordingly the

ranted. The approach to infusion therapy requires increasing assessment in terms of selected components.⁵

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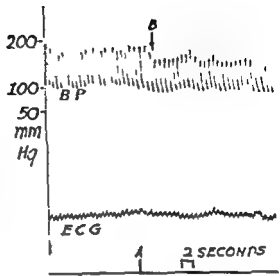
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Fig. 2. Continuous arterial blood pressure and electrocardiographic recordings in a 70 year old woman undergoing cholecystectomy and common bile duct exploration with nitrous oxide ether and curare. At A a hand was inserted in the abdominal cavity. Blood pressure fell within 2 seconds and continued to fall in a stepwise manner. There was no change in the electrocardiogram.



ment of the liver insertion of the hand into the peritoneal cavity and movement from one area to another. Placement of packing, excision and retraction of the wound edges produced the change. Deliberate traction on the mesenteries of bowel and stomach failed to produce hypotension in several cases. Likewise stimulation of the celiac plexus was ineffective. Clamping the gallbladder wall and traction failed, but exploration in the common duct region produced a marked response even with care taken to avoid obstruction of the portal and caval veins. In 9 patients when the venous flow was occluded there was no effect in 1 and minimal changes in the others.

Analysis of the pulse wave contour provided some clues as to the circulatory adjustments involved. In Figure 3 it can be seen that during hypotension there is a flattening of the wave suggesting a decrease in cardiac output rather than a decrease in peripheral resistance. The change in output could be a manifestation of lesser venous filling or decreased cardiac contractility. Additional circulatory measurements are required to establish the specific nature of the changes.

The electrocardiographic changes observed in lead II were by no means as frequent or uniform as the changes in blood pressure. Of the 50 patients studied 17 exhibited auriculoventricular dissociation with nodal rhythm or nodal rhythm alone. These abnormalities were associated with a variety

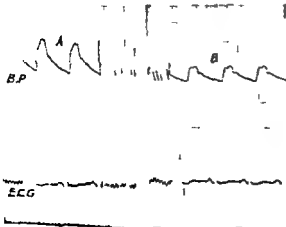


Fig. 3. Tracings from the same patient as in Figure 2 show pressure pulse contours before (A) and after (B) production of hypotension by intraabdominal manipulation. Paper speed has been changed to illustrate the change more clearly. The decrease in pulse pressure occurred without change in the duration of systole or the position of the dicrotic notch. This suggests a decrease in cardiac output rather than change in peripheral resistance.

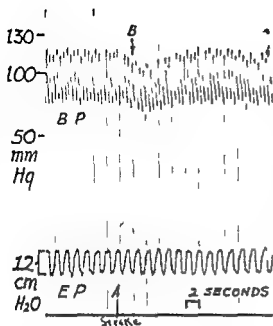


Fig. 1 Continuous arterial blood pressure (upper tracing) and esophageal pressure (lower tracing) in a 52 year old woman undergoing cholecystectomy with nitrous oxide and ether. At A the parietal peritoneum of the upper anterior abdominal wall was stroked with a small ball sponge. Within 2 seconds at B systolic and diastolic pressures fell. The minimum fall was 15 mm Hg systolic and 5 mm Hg diastolic. The duration of the hypotension was 8 seconds. There was no change in esophageal pressure.

surgeon rubbed localized areas of the parietal and visceral peritoneum with a small ball sponge as discretely as possible. Friction on the mesenteries, pressure over the area of the coeliac plexus, traction on the gallbladder and stomach, and occlusion of the portal and caval veins were the other stimuli applied.

RESULTS

In 55 of the 68 patients studied, arterial hypotension was observed in relation to abdominal manipulation. In only 1 was hypertension seen. The latter occurred during upper abdominal exploration in a patient undergoing a pelvic operation under spinal anesthesia. Eleven patients showed a negligible drop in blood pressure. In the remainder, or 44 patients, hypotension was moderate to marked. The manner in which hypotension was produced is interesting. In some cases the change seemed to be clearly reflex in origin. That is, with fairly specific manipulation and little likelihood of interference with venous return to the heart and with no change in the depth of anesthesia or accompanying respiratory phenomena, the blood pressure began to fall within a matter of seconds. A typical response may be seen in Figure 1. The minimum fall was 15 mm Hg systolic and 5 mm Hg diastolic. The pressure began to return before the stimulation was stopped. The fall in pressure lasted 8 seconds. A response of this type occurred with application of towels to the peritoneal margins in the incision or with insertion of the surgeon's hand into the abdominal cavity. In some individuals these stimuli failed to produce the effect. Repeating the stimulus in another area reproduced the same response. The most sensitive area in this regard was the anterior parietal peritoneum of the upper abdomen where is the surface of the liver, stomach and other viscera were insensitive.

In other cases, although a reflex fall in blood pressure seemed likely, contributing factors could not be eliminated. The fall in blood pressure was of greater magnitude, with less tendency to revert to normal. A stepwise drop in pressure shown in Figure 2 was detected in some of the records. Displace-

A SIMPLE METHOD FOR THE PRE TRANSFUSION REMOVAL OF POTASSIUM FROM STORED WHOLE BLOOD*

With observations on the safety of cation exchange collected blood for massive transfusions

ROY H. CLAUS, HILLARY CHOLLET, SALVATORE J. GIANNINI AND
ALEXANDER HENDERSON

The desirability of a simple and inexpensive method of removing excess potassium ions from stored whole blood prior to the transfusion of large quantities is implicit in the high concentrations of potassium known to be present in whole blood stored for several weeks. This study was begun to determine how readily potassium may be removed by passing whole blood through an ion exchange column as the blood is being transfused.

Potassium is removed by incorporating a commercially available ion-exchange column† into the route of transfusion distal to the filter. Blood may flow directly into the patient or it may be stored in reservoirs (see Fig. 1) for subsequent rapid transfusion.

METHOD

Part I The plasma concentrations of potassium, sodium and calcium were determined before and after the passage of fresh blood through the ion-exchange column. Human blood stored in ACD solution for varying periods of time was studied similarly. Additional quantities of potassium as the chloride were added to blood and to water to note the potassium removing capacity of a single column. Then ACD whole blood was passed to observe

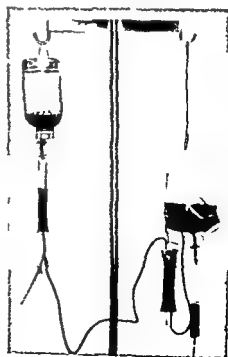


Fig. 1 The ion exchange column blood pack is placed distal to a blood filter in the route of transfusion.

*From the Surgical Service, New York Veterans Administration Hospital and the Department of Pathology, Bellevue Hospital, New York.
†Manufactured by Fenwal Laboratories, 47 Mellen Street, Framingham, Mass.

of factors in most cases, namely deeper levels of anesthesia and abdominal manipulation but almost all with arterial hypotension. Diethyl ether was the common anesthetic agent. Twenty-two patients evidenced a slowing of the pulse rate, 27 showed no change and 6 responded with an increase. A decrease in the amplitude of the T wave and elevation or depression of the ST segment were seen in 25 instances in association with manipulation and hypotension or at other times in the course of anesthesia and operation. In only 1 instance was the change pronounced enough to suggest myocardial ischemia.

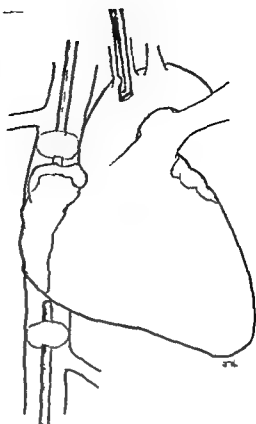
DISCUSSION AND SUMMARY

The common response to intra-abdominal manipulation in these clinical studies was arterial hypotension often associated with bradycardia. The impression gained is that tissue deformation was the basic stimulus for this response, that the parietal peritoneum was far more sensitive than the visceral and that of the upper abdomen particularly so. At the present time we can only speculate about the receptors, the pathways involved and the factors leading to the circulatory alteration. A possible receptor is the Pacinian corpuscle. The afferent pathway might be the intercostal nerves as suggested by Reeve in work on respiration.⁴ Further work in laboratory animals and man will be carried out to assay the role of the phrenic nerve, splanchnics, and vagus nerve in the carriage of afferent impulses from the abdomen. It seems likely that the vagus nerve provides the efferent limb of the reflex arc and that vagal action alone might be responsible for the hypotension observed. The occurrence of bradycardia suggests this and Peterson⁵ has recently suggested that the vagi may influence ventricular contraction.

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Fig. 2. Balloon catheters permit occlusion of venous return to the heart without thoracotomy.



RESULTS

Part II All dogs survived. The control clotting times of 3 minutes rose to 7 to 9 minutes after the experiment. The blood pressure returned to normal after each transfusion upon the deflation of the venae cavae balloons. The dogs' serum electrolytes immediately after the experiments averaged potassium 2.9, sodium 149 and calcium 4.0 mEq/L. One hour post experiment values were 3.2, 150 and 4.5, and 2 hour concentrations were 3.2, 150 and 5.5 mEq/L, respectively.

DISCUSSION

In 1950 C. W. Walter introduced to the Surgical Forum the sulfonated polystyrene copolymer ion exchange column as the basis of a technique for the collection, storage, and administration of unadulterated whole blood.¹ We have used that resin column in these experiments. The original communication placed emphasis upon the decalcifying action of the resin column, thereby rendering blood incoagulable without the addition of substances with detrimental physiological actions, notably citrate. Investigators subsequently have employed ion exchange collected blood in experiments but have added magnesium and potassium before transfusing blood in volumes comparable to the large volumes in the current experiments. While the addition of chemicals to reconstitute normal ion concentrations is reasonable since it may preclude some ion deficiency side-effects, this has been shown to be unnecessary for survival. There is a rapid return of plasma electrolyte concentrations toward normal values to the asymptomatic range.

In 1913 Bruneau and Graham² in 1913 and Cookson³ in 1951 published the results of dog bleeding and retransfusion experiments. All dem-

the volume that would flow through one column without adverse mechanical or osmotic effects. Finally the physiologic effects of the transfusion of large volumes of blood collected *via* the ion exchange column were studied by the biologic responses of 25 dogs.

Blood samples were centrifuged and decanted. The serum or plasma samples were analyzed for sodium and potassium by flame photometry. Calcium was determined by a permanganate method modified after Roe. Fresh whole blood was collected *via* the column. Aliquots were labelled with radiochromate and were reinfused into the donor and into a compatible recipient. The erythrocyte survivals were compared with the normal slope of degradation.

Osmotic fragility of ion column collected red cells was tested.

RESULTS

Part I Our studies have confirmed the presence of high levels of potassium ions in whole blood stored in ACD solution. The plasma concentration parallels the duration of storage, after collection, being of the order of 20 to 25 mEq/l. in 3 week old blood. Fresh blood, 3 week old blood and blood with added potassium (to a concentration of 10 mEq/l.) had the potassium concentrations reduced to 1 mEq/l. after column passage.

The potassium removing effectiveness of a single column was observed to be at least 10 mEq (the equivalent of 25 mEq/l. of potassium in the plasma of 2500 cc. of blood). The normal erythrocyte survival is in agreement with previous studies.⁸ There was no abnormal erythrocyte fragility.

Sodium concentrations rose from 110 (control) to 148 mEq/l. Calcium concentrations in the plasma of fresh blood fell to 1 mEq/l. The plasma calcium levels of ACD collected blood changed little, apparently indicating the citrate calcium bond. (The physicochemical status of the small amount of calcium which was removed by the column is unknown).

METHOD

Part II The freedom from toxic effects of blood collected through an ion exchange column was demonstrated by bleeding and retransfusion experiments in dogs. Morphine sulfate premedication of 3 mg./kg. of body weight was given. Femoral, carotid and external jugular arteries and veins were cannulated, as necessary, using 2 per cent procaine local anesthesia. Systolic blood pressures were read from a mercury manometer. After the experiments the wounds were closed with catgut. The dogs were observed for 4 days to several weeks. Ten dogs were bled and retransfused in increments of 10 per cent to 60 per cent⁹ to a total of 250 per cent of blood volume. In 15 dogs urethral (Foley) catheters were passed to the junction of the superior vena cava with the right atrium and into the inferior vena cava cephalad of the hepatic veins (see Fig. 2). The balloons were inflated with 5 cc. of water to obstruct vena cavae and azygos inflow, demonstrated by a prompt fall in blood pressure to near zero levels. Repeated bleeding of 10 per cent of blood volume was followed by supra-aortic infusion at 14 mm./Hg. pressure after inflation of the catheter balloons produced cardiac inflow occlusion. This was repeated to a total exceeding 250 per cent of the blood volume. All bleeding and retransfusion was accomplished in 30 to 90 minutes.

⁹10 per cent of body weight was used as the weight of the total blood volume.

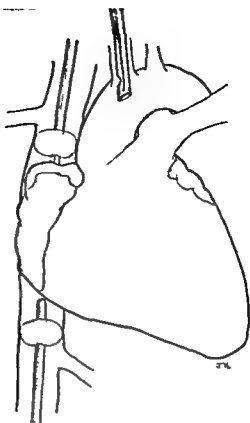


Fig. 2 Balloon catheters permit occlusion of venous return to the heart without thoracotomy

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Ivy³ in 1943, Bruneau and Graham² in 1943, and Cookson² in 1954 published the results of dog bleeding and retransfusion experiments. All dem-

onstrated the marked toxicity of citrated blood contrasted with minimal lethality of heparin. The latter introduced prolonged clotting times. The experiments reported in the present paper were devised to make similar observations while bleeding greater total volumes in less time using only the ion exchange blood. Two distinct advantages of the use of ion exchange blood compared with heparin are the permanent anticoagulation and the minimal derangement of clotting times in the recipient.

The preceding statements have referred to unadulterated whole blood (non citrated). It is to be noted that the citrated whole blood passed through the ion exchange column to remove excess potassium must be assumed to retain its qualities of citrate toxicity. The cautions of Adams¹ against transfusing citrated blood at a rate exceeding 1 liter per hour must be remembered as well as the observations of others of the hazards of even smaller quantities of citrated blood in patients with liver disease and in cardiac and pediatric patients.

It is recognized that not all instances of acute renal failure will develop hyperkalemia. However it is equally certain that the cause of death in renal failure appears to be related to hyperkalemia in many instances. Tissue destruction, stress, hypovolemic shock and the rapid transfusion of large quantities of blood coincide with elevated plasma potassium. The re warming of blood prior to transfusion to aid the movement of potassium from the plasma into the erythrocytes is time consuming and uncertain. Glucose, insulin, testosterone and digitalis therapy are best reserved for the unavoidable endogenous potassium accumulations in the extracellular space. To the patient's advantage excessive exogenous plasma potassium may be kept from the blood recipient simply and inexpensively in the transfusion process described herein.

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ETIOLOGY AND PREVENTION OF SLOUGHS PRODUCED BY L NOR EPINEPHRINE*

JACQUES Y. BIRNEN, MILTON F. BRYANT, AND JOHN M. HOWARD

A distressing and not infrequent complication associated with the administration of a continuous intravenous drip of l nor epinephrine has been the production of extensive soft tissue sloughs. In an effort to clarify the etiology of nor epinephrine sloughs and to determine how these sloughs could be prevented the following studies have been carried out.

METHOD

The studies were performed on mongrel dogs using a test solution of 5 per cent glucose in water containing $8 \mu\text{g}$ l nor epinephrine cc.

Etiology. At the onset of this study it was recognized that subcutaneous infiltration of nor epinephrine would produce soft tissue sloughs.¹⁻³ Various authors¹⁻⁴ have also suggested that sloughs may result from the intravenous administration of solutions containing nor epinephrine. The current report deals with the local effects of the drug when administered intravenously or subcutaneously. Studies include observations on (1) The local effects of the intravenous injection of nor epinephrine test solution when the solution is administered to normal dogs as follows: a) Rapid injection of 10 cc of test solution into the venae cavae and femoral veins in 5 dogs; b) Rapid injection of 10 cc of test solution into medium sized veins in 5 dogs; c) Drip injection (50-70 minutes) of 100 cc of test solution into the venae cava and femoral veins in 5 dogs; d) Continuous intravenous drip injection for periods up to 72 hours into medium sized veins in 5 dogs.

(2) The local effects of l nor epinephrine test solution when given intravenously to dogs in hemorrhagic shock and to dogs whose inferior vena cava had been ligated were studied in 6 dogs.

(3) The local effects of the subcutaneous injection of l nor epinephrine test solution were studied by: a) Rapid injection of 100 cc of test solution into the subcutaneous tissues in 12 dogs; b) Subcutaneous drip injection (50-70 minutes) of 100 cc of test solution in 12 dogs.

Prevention of sloughs produced by nor epinephrine. Once a standard preparation was achieved that would permit the uniform production of a sloughing wound following the subcutaneous drip infusion of nor epinephrine prevention of the necrotizing effect was attempted as follows: (1) Infusion subcutaneously of saline histamine procaine hyaluronidase and papaverine in respective experiments following the extravasation of nor epinephrine in 10 dogs. Effect of perivascular sympathectomy immediately after subcutaneous extravasation of nor epinephrine in 2 dogs. (2) Effect of Regitine on the subcutaneous extravasation of nor epinephrine: a) Subcutaneous drip injection (50-70 minutes) of 100 cc of test solution followed by immediate intravenous injection of 10 mg of Regitine in 5 dogs; b) Subcutaneous drip injection of 100 cc of test solution followed by immediate and delayed (1-2

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3 4 6 10 12 16 18 20 and 24 hours) injection of 10 mg of Regitine into the area of nor epinephrine extravasation in 15 dogs

OBSERVATIONS

Etiology. Intravenous infusion The direct local effect associated with the intravenous administration of nor epinephrine test solution into medium sized veins was the production of marked venous spasm of the vein receiving the injection or drip infusion. The spasm or contraction lasts from 4 to 6 hours and then disappears even though the drip infusion is continued. Spasms of large veins (femoral and vena cava) could not be produced with the nor epinephrine test solution. Leakage of the test solution through the vein wall was not observed even though the drip injection was continued for periods up to 72 hours. Studies performed on dogs in hemorrhagic shock and on dogs with their vena cava ligated did not differ from the results obtained in normal dogs.

Subcutaneous infusion Rapid injection of 50 to 100 cc of test solution into the subcutaneous tissues did not produce soft tissue necrosis; however the infusion of the same quantity of test solution into the subcutaneous tissues over a 30-60 minute period routinely produced tissue necrosis. Concentration of nor epinephrine was not important as 4 to 8 cc of concentrated nor epinephrine was injected subcutaneously without producing sloughs.

Prevention of sloughs produced by nor epinephrine Saline histamine procaine hyaluronidase papaverine and sympathectomy were not effective in preventing sloughs produced by extravasation of the nor epinephrine solution. Regitine injected into and about the margins of the subcutaneous infiltration of nor-epinephrine test solution routinely prevented the formation of soft tissue sloughs up to periods of 12 hours following extravasation of the test solution (See Fig 1). Prevention of sloughs by Regitine after the lapse of 12 hours was unpredictable; however some effect was noticed 18 hours following the infiltration of the nor epinephrine test solution. Regitine did not affect the outcome of tissue necrosis if injected 18 or more hours following the extravasation.

DISCUSSION

In spite of clinical experience we have found no evidence that the intravenous administration of nor epinephrine will produce soft tissue sloughs. Drip infusion of solutions containing nor epinephrine produce marked con-



Fig. 1 Right thigh—effect of extravascular infiltration of nor epinephrine. Left thigh—prevention of tissue necrosis by injecting Regitine into the nor epinephrine extravasation.

traction of medium sized veins receiving the infusion. This spasm lasts from 1 to 6 hours and then is replaced by venous dilatation even though the infusion is continued. This would seem to rule out the possibility that prolonged spasm of veins in the region of a drip infusion of nor epinephrine might cause tissue necrosis as suggested by Kurlind *et al.*³ and Uricchio *et al.*⁴ Humphries and associates⁵ postulated that spasm of the vas vasorum might interfere with the nutrition of the walls of the vein and lead to increased permeability and extravasation. We could not demonstrate although we could entirely rule out leakage through the walls of veins receiving nor epinephrine even though the test solution was given continuously for periods up to 72 hours. Skin necrosis did not occur in the absence of extravasation.

Wall and Hinton⁶ suggested that cutaneous ischemia induced by shock would be aggravated by nor epinephrine and lead to tissue necrosis. In the current experiments soft tissue sloughs did not result from the intravenous administration to dogs in hemorrhagic shock.

Bergmann⁴ suggests that the hydrostatic pressure of the drip infusion of nor adrenaline was much higher than the venous pressure so that the nor adrenaline passed *via* retrograde flow through the tributary veins to capillaries and arterioles. Intense spasm of regional capillaries and arterioles then occurred which led to ischemia and gangrene. Even with the inferior vena cava ligated so as to force the nor epinephrine solution into the tributary veins soft tissue sloughs could not be produced.

The rapid injection of 50 to 100 cc of the test solution into the subcutaneous tissues failed to produce sloughs. The same concentration and quantity of nor epinephrine infused into the subcutaneous tissues over a period of 30 to 60 minutes routinely produced soft tissue sloughs. Why this occurred is not readily explainable.

Regitine (Phentolamine Liba) is dramatically effective in preventing tissue necrosis by solutions containing nor epinephrine if injected into the extravasated area immediately or up to 12 hours following extravasation. Between 12 and 18 hours it was found that the extent of tissue necrosis was definitely diminished. Regitine did not affect the outcome of tissue necrosis if injected 18 or more hours following extravasation. The action of Regitine appears to be local as Regitine injected intravenously or into the subcutaneous tissues in the leg opposite the extravasation will not prevent tissue necrosis. Regitine is so powerful in its action that it presumably prevents the action of nor epinephrine upon the smooth muscle cells. Other potent pharmacologic agents (procaine, hyaluronidase, papaverine and histamine) were not effective in preventing soft tissue sloughs produced by extravasated solutions containing nor epinephrine.

SUMMARY

- 1 Soft tissue sloughs associated with the intravenous administration of solutions containing nor epinephrine are due to extravascular infiltration of the solution.
- 2 Soft tissue sloughs can be prevented by preventing the extravasation of solution containing nor epinephrine.
- 3 If extravasation inadvertently occurs tissue necrosis can be minimized or prevented by diluting 10 mg of Regitine in 20 cc of saline and injecting this solution into and about the margins of the extravasation.

% 4, 6 8 10 12, 16 18 20 and 21 hours) injection of 10 mg of Regitine into the area of nor epinephrine extravasation in 15 dogs

OBSERVATIONS

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Fig. 1 Right thigh—effect of extravascular infiltration of nor epinephrine. Left thigh—prevention of tissue necrosis by injecting Regitine into the nor epinephrine extravasation.

3.0 to 4.0 cm within the pulp of the lower splenic pole. The manometer bases were placed at the level of the branch vein or venostatic level.

In the Group 2 series a #13 or more preferably a #15 gauge needle was introduced through the left 9th or 10th costal interspaces in the mid or posterior axillary line. With the manometer base at the hub of the needle pressures were read at splenostatic level. Three observations of the leveling meniscus were recorded and averaged to the nearest centimeter. No calibration was interposed for the mean anatomical level of the portal vein or the right atrium.

Simultaneous recordings at venostatic and splenostatic levels indicated that the former is 10.0 to 12.0 cm higher than the latter or the anatomical difference in manometer bases. This factor is not interposed but must be considered when interpreting our results. The fluid medium was normal saline with 20 mg of heparin sodium added to each 100 cc.

The 15 control patients had no evidence of portal venous disease and were usually undergoing gallbladder or gastric surgery. The simultaneous portal and pulp manometries were taken at venostatic level upon opening the abdomen with the peritoneal cavity free of surgical instruments.

The 40 patients in Group 2 were subdivided. *Subgroup A* was composed of 32 patients with clinical and laboratory evidence to support the diagnosis of cirrhosis. Twenty nine had liver biopsies which were reported as follows: Laennec's cirrhosis 21, periportal fibrosis 1, fatty changes 2, biliary stasis and cholangitis 1, and biliary cirrhosis 1. For the purposes of the current study all were considered cirrhotics. In 6 the livers were nonpalpable.

Subgroup B consisted of 6 patients with liver biopsies confirming metastatic disease. Two patients who were placed in a miscellaneous group had diagnoses of congenital hemolytic jaundice and myelogenous leukemia with hypersplenism.

The Group 2 studies were performed in the x-ray department with the patients supine on the Sanchez Perez Automatic Sierograph which produced 8 exposures at one-second intervals. Local anesthesia was used in all but 6 instances while the contrast material injected was 30 cc of Urokon (R) sodium 70 per cent (sodium acetrizate).

All but 4 patients in *Subgroup A* were esophigoscoped while esophigograms were routinely performed. Two of the 4 unscooped individuals had definite evidence of varices on portography. Splenomegaly was determined by palpation only though in at least 3 patients it appeared enlarged on scout films. Standard liver function studies were repeatedly performed on all patients.

The *Subgroup A* results were examined with respect to ascites, jaundice, splenomegaly, esophago-gastric varices and gastrointestinal hemorrhage.

The portograms were rated as *normal*, *abnormal* or *unsatisfactory*. In the normal film the splenic vein was of small caliber (1.0 cm or less in diameter), transverse in position and with the exception of the portal the only vein visualized. For an abnormal rating or anatomical evidence of hypertension 2 or more of the following were necessary: a large tortuous splenic vein (1.0 cm or more in diameter), identifiable coronary, short gastric, mesenteric or perisplenic veins or varices. Preferential flow through collaterals was considered pathognomonic of elevated tension and more than an 8 second delay in solid opacification of the liver was considered obstructive.

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SPLENIC PULP MANOMETRICS*

*Preliminary observations of an apparently simple method
for measuring portal venous pressure*

FRANCIS C JACKSON AND JOHN L HAMIL

Recently, four new techniques were reported for direct and indirect evaluation of portal venous pressure in the unanesthetized state: 1) by catheter wedged hepatic vein¹ - by cannulation of the portal vein, transhepatically² or transabdominally³ and by the percutaneous needle puncture of the splenic pulp.⁴

The current manometric study was suggested by Sherlock and others who demonstrated that the pulp in splenomegalic patients possessed a labile measurable tissue pressure which correlated with wedged hepatic pressures and estimated portal pressures with minimal gradient. Splenic pulp pressures and percutaneous portography offered a new combined method of assessing portal circulation.

Our study sample consisted of 61 splenic punctures and 31 portograms performed upon 55 hospitalized patients. With the exception of a control group patients with disease of the portal circulation were selected for study.

All pressures were recorded on the standard spinal fluid manometer calibrated to 60.0 cm (B&D#5027). The technique of Child⁵ was used in the Group I or control series to record simultaneous pulp and portal vein pressure measurements. A #19 polyethylene catheter through a major branch was introduced into the portal vein while a #13 gauge needle was placed

*From the Veterans Administration Hospital and the Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh 12.

equal or 10.0 cm. In 11 studies there was little or no gradient. Where a reverse gradient seemed present, i.e. the portal greater than the pulp pressure by more than 1.0 cm, in 2 patients we suspected that there were unrecognized technical errors.

Table 1 Splenic Pulp Pressure at Splenostatic Level in Group 2

I SUBGROUP A (CIRRHOSIS)	CLINICAL SIGNS	NO PATIENTS EXAMINED	MEAN PULP PRESSURE (Cm H ₂ O)	EXTREMES (Cm H ₂ O)
Mean pressure in 32 patients— 33.0 cm	1 Splenomegaly	19	40.0	30.0—58.0
	2 Asplenomegaly	13	27.0	11.0—31.0
	1 Varices	16	40.0	30.0—58.0
	2 No varices	16	27.0	14.0—43.0
	1 G I Hemorrhage	17	33.0	16.0—52.0
	2 No G I Hemorrhage	15	32.0	14.0—58.0
	1 Hemorrhage from varices	10	40.0	31.0—52.0
	2 Hemorrhage from other sources	7	26.0	10.0—32.0
	1 Jaundice (serum bilirubin >2.5 mg %)	11	31.0	16.0—52.0
	2 No jaundice (serum bilirubin <2.5 mg %)	21	33.0	11.0—58.0
	1 Ascites	8	33.0	20.0—58.0
	2 Ascites and jaundice	9	37.0	31.0—52.0
II SUBGROUP B (LIVER METASTASES)				
Mean pressure in 11 patients— 27.0 cm	1 Hepatomegaly	5	28.0	12.0—38.0
	2 Hepatomegaly & splenomegaly	2	32.0	20.0—38.0
III MISCELLANEOUS				
	1 Congenital hemolytic jaundice	1	27.0	
	2 Myelogenous leukemia	1	0.0	

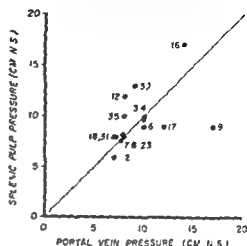
Group 2. The results of related clinical data and pulp pressures are tabulated in Table 1.

There appeared to be a definite relation between the clinical stigmata of

liver disease. Due in the subcapsular space or poor opacification of the portal system was an unsatisfactory study

Fig. 1 The manometer base levels were located at the level of the cannulated portal branch vein or the "venostatic level" in the abdominal wound. In 9 of the 15 studies the pulp pressure was within 10 cm of the portal. Where a difference of more than 10 cm existed as in 7 a pulp gradient was noted in 6. There was little or no gradient in 11 observations.

SIMULTANEOUS SPLENIC PULP AND PORTAL VEIN PRESSURES AT WOUND LEVEL IN 15 CONTROL PATIENTS AT LAPAROTOMY



RESULTS

Group 1 Figure 1 is a plot of the 15 simultaneous uncorrected portal and pulp pressures at venostatic level. The means of the 2 pressures were

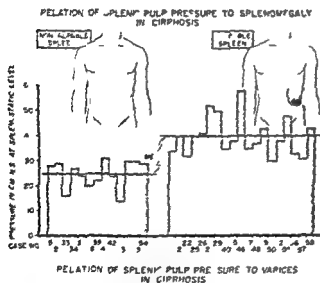


Fig. 2 There were no palpable spleens in 10 patients with pulp pressures below 29.0 cm while 10 of 22 spleens were palpable with manometrics of 30.0 cm or over

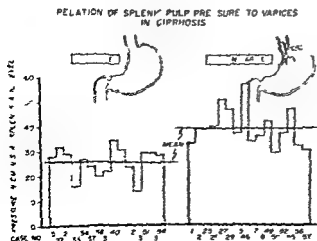


Fig. 3 Varices were present in 16 of 22 patients with pulp pressures over 30.0 cm and 8 of 9 over 40.0 cm. All with varices had spleno megal

equal or 10.0 cm. In 11 studies there was little or no gradient. Where a reverse gradient seemed present, i.e. the portal greater than the pulp pressure by more than 1.0 cm, in 2 patients we suspected that there were unrecognized technical errors.

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Mean pressure in 6 patients— 27.0 cm	1 Hepatomegaly	5	28.0	12.0—38.0
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III MISCELLANEOUS				
	1 Congenital hemolytic jaundice	1	27.0	
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Group 2. The results of related clinical data and pulp pressures are tabulated in Table 1.

There appeared to be a definite relation between the clinical stigmata of

portal hypertension and the degree of splenic pulp pressure (Fig 2) Splenomegaly apparently develops or is almost always present with pulp pressures over 30.0 cm. When the 35.0 cm level is reached both varices and the large spleen are usually demonstrable. Varices were present in only 4 of 18 below 35.0 cm (Fig 3).

From these observations there does not appear to be a very definite relationship of pulp pressure to serious parenchymal damage in cirrhosis although when ascites and jaundice were associated with splenomegaly and varices the pulp tension was correspondingly elevated.

The mean of 17 patients with hemorrhage was 35.0. However when the bleeding proved to be from varices the mean was 40.0 cm.

Apparently it may be impossible to record a valid tissue pressure in a cellular disease which involves the pulp. We obtained no pressure in a huge spleen of a patient with myelogenous leukemia.

Three patients were studied before and after end to side porta caval shunt. Two of the pulp pressures were reduced from pulp tensions of 40.0 and 52.0 cm to 17.0 and 26.0 cm respectively. One pressure of 35.0 cm was 38.0 cm postoperatively. This caused some concern regarding the patency of the shunt until the portogram demonstrated an incomplete caval compression by the liver. The pressure in the inferior vena cava had been 24.0 cm and the narrow gradient across the shunt was a source of concern at surgery.

There were 26 portograms sufficiently satisfactory for evaluation in *Sub group A*. Twenty three were abnormal by our definition. The mean pulp pressure was 35.0 cm with extremes of 22.0 and 58 cm. Only 5 abnormal portograms were noted with pressures below 30.0 cm. The mean of the 3 normals was 28.0 cm.

With metastatic tumor in the liver portograms usually demonstrated the vascular nodules. A tremendous collateralization was produced between the splenic and bifid renal veins where the splenic vein was invaded in 1 patient. This pulp pressure was 32.0 cm.

DISCUSSION

A free communication between the pulp spaces or sinusoids and the richly anastomotic intrasplenic venules has been given as the principal anatomical relation between the spleen and the portal circulation. Its intimate relation with the splanchnic system marks it as an integral part in rather than a dependent organ of the general circulation.

While much study remains it would seem that the pulp pressure can be a reliable index of portal pressure. Clinical support for this contention would seem to be the relation of this tissue pressure to splenomegaly and varix formation.

When the pulp tissue pressure exceeds 35.0 cm of saline at splenostatic level and is associated with collateralization on portogram gastrointestinal hemorrhage could be anticipated.

There are definite factors of morbidity in this study which should not be minimized. Early in our work it was necessary to remove 2 spleens in Group 1 and 1 in Group 2. We have ascribed these to misguided effort and poor technical judgment. In Group 2 slight or no pain was noted during or following the study in 26 patients; severe pain in 6; fever in 6; hypotension in 3; fainting and requiring transfusions 1 each.

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PLASMIN LYSIS OF EXPERIMENTAL RADIOACTIVE PULMONARY EMBOLI, WITH CONSIDERATION OF A PLASMIN INHIBITION TEST*

MICHAEL HUME WILLIAM W L GLENN PAUL J ROSENBAUM
PAUL H GUILFOIL AND DANIEL L KLINE

Previous work in this laboratory by Scott† and Glenn in which lysis of experimental clots using plasmin was attempted demonstrated a need for detecting clot lysis as it progressed. A method was devised by which the dissolution of a radioactive clot the cells of which were tagged with chromium⁵¹ was studied by sampling the blood for changes in radioactivity during lysis. Somewhat similar methods have been reported elsewhere previously.¹⁻³ In our earlier work the radioactive clot was introduced into the arterial circulation and in these experiments considerable fragmentation of the clot occurred. Furthermore it was difficult to assess the final result by autopsy because any remaining fragments of clot were scattered into a large capillary bed and were unavailable for evaluation. Some of these difficulties were met by introducing the clot into the venous side of the circulation particularly into the inferior vena cava from which it was allowed to pass into the right side of the heart and from there to the pulmonary artery and its branches. The slower circulation of the venous side tended to disturb the clot less and examination of the

From the Departments of Surgery Radiology and Physiology Yale School of Medicine. This work was supported in part by a grant from the American Heart Association and in part by a grant from the Victoria Fund for Cardiovascular Research at Yale University. The fraction III from which the plasminogen used in this investigation was purified was released through the courtesy of Dr S I Gibson and Dr J N Ashworth of the American National Red Cross. Streptokinase was generously supplied by Dr J M Ruegg, segger of Lederle Laboratories division of the American Cyanamide Company.

*Scott F B Senior thesis in partial fulfillment of requirements for M D degree 1955

major branches of the pulmonary artery at autopsy was relatively easy. The present study considers first the loss of substance from a standard radioactive clot after it is introduced into the dog and second the effectiveness of plasminolysis of these experimental clots. Changes in the clotting mechanism are discussed and a plasmin inhibition test described.

METHODS

Preparing the standard radioactive clot. Prior to the cannulation of the inferior vena cava blood was withdrawn for the formation of the radioactive clot. Fifteen ml. was placed into each of 2 bottles containing 4 ml. of ACD solution. Sodium radiochromate (Rachromate, Abbott) was introduced into 1 of these, the amount varying from 0.15 to 0.5 mc. The blood was incubated for 1 hour at 37°C. with occasional gentle rotation of the bottle. The plasma was then withdrawn and discarded and the remaining cells were washed 3 times with cold isotonic saline solution to remove unbound sodium radiochromate. The tagged cells were resuspended in plasma obtained from the second bottle. This blood was introduced into a glass tube which had been rinsed with a strong thrombin solution (Topical Thrombin, Upjohn). Clotting quickly occurred and retraction was allowed to proceed at 37°C. for 1 hour. Loose cells and serum were then removed by washing saline solution past the clot. In a second washing technique the clot was placed on a Buchner filter paper and was washed with the aid of suction. A third method employed a circulating chamber with connecting tubing that passed through a Sigmamotor pump. The simplest and most rapid method proved to be that using the Buchner funnel. The clot was weighed after washing and then floated off the filter paper and reintroduced into the glass tube for injection into the inferior vena cava.

Introducing the clot. The dog was anesthetized with nembutal. Through a transperitoneal incision the vena cava was cannulated cephalad with a wide diameter glass cannula. The glass tube containing the clot was attached to the cannula in the cava and the clot was gently flushed into the vein. The cava was ligated when the cannula was removed and the wound closed tightly. As the animal was sacrificed at the conclusion of the experiment aseptic technique was not observed. Polyethylene cannulae (PE 205, Clay Adams) placed in the femoral artery and right heart (*via* the jugular vein) allowed monitoring of arterial and central venous pressures during embolization.

Preparation and dosage of plasmin. Human plasmin prepared by the method of Kline was used (This preparation contained 1 mg. of protein nitrogen/ml. of solution). In most of the experiments 0.5 ml. containing 0.5 mg./kg. of body weight was given usually as a single dose injected into the catheter that lay in the superior vena cava. The injection was given over a one minute period. Occasionally a second injection was given. The activity of the enzyme was tested against a standard clot for each new batch of plasmin prepared. In every instance the enzyme conformed to the standards of activity as described by Kline.

Plasmin inhibition test. The test as performed is generally comparable to the test used by Tillett, Johnson and McCarty³ to measure streptokinase inhibition in patients using clots of their whole blood except that the enzyme used here was plasmin instead of streptokinase. Serial halving dilutions of

human plasmin were made in 12 x 75 mm test tubes beginning with full strength plasmin (1 mg/ml) and carried to 9 tubes (1/256 plasmin). Each tube contained 0.1 ml of (diluted) enzyme. Into each 1 ml of dog's blood was placed and mixed by tipping the tube. With practice the procedure could be completed within 1 minute and before clotting had occurred. The tubes were placed in a 37°C water bath and a note made of the time of clotting and subsequently the time of complete lysis. The end point was taken as the last tube in which complete lysis occurred and was reported as the dilution of enzyme i.e. 1/2, 1/4, etc. The end point was reached in from 20 minutes to 1 hour. It was read at the last tube with complete lysis whether or not any clot remained in a tube with a higher concentration of plasmin.

Determination of the progress of clot lysis. After the wound was closed a probe type scintillation counter was positioned over the precordium at the point of maximum radioactivity. This was monitored by a Leeds Northrup recorder via a scaler and count rate meter. Two milliliter samples of blood were taken at frequent intervals during the experiment and placed in 10 x 75 mm tubes. Each of these tubes was subsequently counted in a well type scintillation counter. Final estimate of the completeness of lysis was by autopsy with careful dissection of the major branches of the pulmonary artery. In some cases any remaining fragments were collected and weighed.

Studies of clotting factors. Coagulation time of the whole blood was determined in 10 x 75 mm tubes by tilting a 2 ml sample at 1 minute intervals until a solid invertible clot occurred. This was carried out at room temperature. A rapid estimation of fibrinogen was carried out in some cases. The method used was essentially that described by Schneider⁴ except that in some instances Ringer's solution was replaced as a source of calcium by a Veronal isotonic saline buffer containing 0.0025 M calcium chloride. Paper electrophoresis of plasmin in a Spinco Durrum cell was performed in some experiments to demonstrate the presence or absence of fibrinogen. Veronal buffer at pH 8.6 ionic strength 0.1 was used with heparin added to the buffer to prevent clotting. Running time was 16 hours at 7 milliamperes per 8 strips and the strips were then stained with bromphenol blue.*

RESULTS

The *in vitro* loss of radioactivity from the standard clot. Four standard clots were placed for varying periods in a bath of dog serum at 37°C. In 1 instance the serum was circulated through a circulation chamber with a Sigmamotor pump. These 4 clots were reduced to about 30 per cent of their starting weight and the serum in which they lay gained radioactivity. Hemolysis was negligible, the increased radioactivity of the wash serum being present in detached triggered erythrocytes. Most of this change in clot weight occurred in the first 6 hours. The clot was about the same length and shape but was noticeably thinner. It did not tend to fragment. Counts per gram of dry clot weight determined by desiccating each clot was about equal for all samples. Water accounted for 70 per cent of the mass of clot weighed after 6 hours or more and 72 per cent of the mass of clot which was desiccated immediately after initial washing.

*J. J. Barboriak of the department of nutrition Yale School of Medicine did the paper electrophoresis.

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METHODS

Preparing the standard radioactive clot. Prior to the cannulation of the inferior vena cava blood was withdrawn for the formation of the radioactive clot. Fifteen ml. was placed into each of 2 bottles containing 4 ml. of ACD solution. Sodium radiochromite (Rachromite, Abbott) was introduced into 1 of these, the amount varying from 0.15 to 0.5 mc. The blood was incubated for 1 hour at 37°C. with occasional gentle rotation of the bottle. The plasma was then withdrawn and discarded, and the remaining cells were washed 3 times with cold isotonic saline solution to remove unbound sodium radiochromite. The tagged cells were resuspended in plasma obtained from the second bottle. This blood was introduced into a glass tube which had been rinsed with a strong thrombin solution (Topical Thrombin, Upjohn). Clotting quickly occurred and retraction was allowed to proceed at 37°C. for 1 hour. Loose cells and serum were then removed by washing saline solution past the clot. In a second washing technique the clot was placed on a Buchner filter paper and was washed with the aid of suction. A third method employed a circulating chamber with connecting tubing that passed through a Sigmamotor pump. The simplest and most rapid method proved to be that using the Buchner funnel. The clot was weighed after washing and then floated off the filter paper and reintroduced into the glass tube for injection into the inferior vena cava.

Introducing the clot. The dog was anesthetized with nembutal. Through a transperitoneal incision the vena cava was cannulated cephalad with a wide diameter glass cannula. The glass tube containing the clot was attached to the cannula in the cava and the clot was gently flushed into the vein. The cava was ligated when the cannula was removed and the wound closed tightly. As the animal was sacrificed at the conclusion of the experiment aseptic technique was not observed. Polyethylene cannulae (PE 205, Clay Adams) placed in the femoral artery and right heart (via the jugular vein) allowed monitoring of arterial and central venous pressures during embolization.

Preparation and dosage of plasmin. Human plasmin prepared by the method of Kline² was used. (This preparation contained 1 mg. of protein nitrogen/ml. of solution.) In most of the experiments 0.5 ml. containing 0.5 mg./kg. of body weight was given usually as a single dose injected into the catheter that lay in the superior vena cava. The injection was given over a one minute period. Occasionally a second injection was given. The activity of the enzyme was tested against a standard clot for each new batch of plasmin prepared. In every instance the enzyme conformed to the standards of activity as described by Kline.

Plasmin inhibition test. The test is performed in generally comparable to the test used by Tillett, Johnson and McCarty³ to measure streptokinase inhibition in patients using clots of their whole blood except that the enzyme used here was plasmin instead of streptokinase. Serial halving dilutions of

human plasmin were made in 12 x 75 mm test tubes beginning with full strength plasmin (1 mg./ml) and carried to 9 tubes (1/256 plasmin). Each tube contained 0.1 ml of (diluted) enzyme. Into each 1 ml of dog's blood was placed and mixed by tipping the tube. With practice the procedure could be completed within 1 minute and before clotting had occurred. The tubes were placed in a 37°C water bath and a note made of the time of clotting and subsequently the time of complete lysis. The end point was taken as the last tube in which complete lysis occurred and was reported as the dilution of enzyme i.e. 1/2, 1/4, etc. The end point was reached in from 20 minutes to 1 hour. It was read at the last tube with complete lysis whether or not any clot remained in a tube with a higher concentration of plasmin.

Determination of the progress of clot lysis. After the wound was closed a probe type scintillation counter was positioned over the precordium at the point of maximum radioactivity. This was monitored by a Leeds Northrup recorder via a scaler and count rate meter. Two milliliter samples of blood were taken at frequent intervals during the experiment and placed in 10 x 75 mm tubes. Each of these tubes was subsequently counted in a well type scintillation counter. Final estimate of the completeness of lysis was by autopsy with careful dissection of the major branches of the pulmonary artery. In some cases any remaining fragments were collected and weighed.

Studies of clotting factors. Coagulation time of the whole blood was determined in 10 x 75 mm tubes by tilting a 2 ml sample at 1 minute intervals until a solid invertible clot occurred. This was carried out at room temperature. A rapid estimation of fibrinogen was carried out in some cases. The method used was essentially that described by Schneider⁴ except that in some instances Ringer's solution was replaced as a source of calcium by a Veronal isotonic saline buffer containing 0.0025 M calcium chloride. Paper electrophoresis of plasma in a Spino Durrum cell was performed in some experiments to demonstrate the presence or absence of fibrinogen. Veronal buffer at pH 8.6 ionic strength 0.1 was used with heparin added to the buffer to prevent clotting. Running time was 16 hours at 7 milliamperes per 8 strips and the strips were then stained with bromophenol blue.*

RESULTS

The *in vitro* loss of radioactivity from the standard clot. Four standard clots were placed for varying periods in a bath of dog serum at 37°C. In 1 instance the serum was circulated through a circulation chamber with a Sigmamotor pump. These 4 clots were reduced to about 30 per cent of their starting weight and the serum in which they lay gained radioactivity. Hemolysis was negligible; the increased radioactivity of the wash serum being present in detached ragged erythrocytes. Most of this change in clot weight occurred in the first 6 hours. The clot was about the same length and shape but was noticeably thinner. It did not tend to fragment. Counts per gram of dry clot weight determined by desiccating each clot was about equal for all samples. Water accounted for 70 per cent of the mass of clot weighed after 6 hours or more and 72 per cent of the mass of clot which was desiccated immediately after initial washing.

* J. J. Barboriak of the department of nutrition, Yale School of Medicine did the paper electrophoresis.

Table 1 Results of plasmin lysis of experimental radioactive pulmonary emboli including autopsy evaluation, changes in radioactivity, plasmin dosage and changes in the clotting mechanism

TIME AT AUTOCLAVE	CLOT LYSIS AT AUTOCLAVE	CHANGE OF RADIOACTIVITY (COUNTS PER MINUTE IN THOUSANDS) 1 RECORDING	DOSE OF PLASMIN (MG/AG)	WOUND OOZE	DURATION IN COAG OF BLOOD (HR.)	CHANGE IN FIBRINOGEN TITER
11 30	complete	14 to 12	20 to 22	slight	0	1 50 to 6
11 48	complete	10.5 to 7.7	11.5 to 12.1	none	3	1 200 to 6
11 27	equivocal	2.5 to 2.3	9 to 10	none	never	1 100 to 1 10
11 22	about half	2.5 to 1.5	9 to 1.5	yes	3	1 100 to 6
11 21	more than half	3.2 to 1.9	8 to 1.8	yes	4	1 100 to 6
11 19	more than half	2.9 to 1.8	5 to 1.1	yes	4	1 50 to 6
11 17	less than half	3.1 to 2.7	1.5 to 1.9	yes	4	
11 16	complete	2.1 to 1.2	4 to 2	none	never	
11 15	about half	6.7 to 5.2	3.3 to 5.1	none	never	
11 11	complete		2.6 to 2.9	yes	8	returning at 8 hr
11 13	complete	1.8 to 2.8	0.7 to 3.3	yes	1	
11 12	three quarters	6.1 to 3.2	2 to 1.1			
11 11	three quarters	4.1 to 2.4	7 to 1.5		3	
11 10	equivocal	8 to 5	5 to 5			
11 9	more than 3/4	1.4 to 4	3 to 2.6		4	
11 8	more than 3/4	1.1 to 1.3	1.2 to 5		3	

*1 Plasminogen 4 mg and streptokinase 4 mg/kg.

Hemodynamic changes due to embolization Venous and arterial pressure tracings taken during introduction of the clot into the inferior vena cava showed changes suggestive of pulmonary embolization in some experiments. In these cases the femoral artery pressure fell and the central venous pressure rose sharply. These changes usually returned to previous levels in 2 to 4 minutes. In only 2 of 21 experiments using the standard clot did the dog expire as a result of acute pulmonary embolization.

Lysis using plasmin The results of plasmin lysis are presented in Table 1. Of the 16 experiments, complete lysis was achieved in 5 and three quarters lysis or more in an additional 1. Five cases showed lysis of about half of the clot. In 2 experiments there was little or no evidence of clot lysis.

Changes in the coagulability of the blood In most experiments in which clot lysis occurred, the blood became incoagulable immediately after the enzyme was given and shortly thereafter the wound began to ooze. Fibrinogen levels, where determined, fell to zero as measured by the rapid assay method and paper electrophoresis showed the fibrinogen band to be absent. With the blood incoagulable, 0.5 ml. of blood mixed with 1.0 ml. of blood from a normal animal gave a solid clot which might undergo partial lysis after several hours. Table 1 indicates the period during which the blood was incoagulable, presence of wound ooze, and the results of fibrinogen determinations when these were done.

Plasmin inhibition tests Seventeen dogs have been used in tests to demonstrate inhibition of human plasmin activity in clots of their whole blood. Complete lysis to the fifth tube (plasmin 1:16) was the maximum fibrinolytic effect (the least inhibition). No lysis or lysis in the first tube only (maximum inhibition) occurred in 5 dogs. Lysis to the fourth or fifth tube (1:8 or 1:16) was recorded in 7 animals. Results varied from day to day for the same animal by 1 or 2 tubes and were uniformly very low (maximum inhibition) under anesthesia.

DISCUSSION

The method for preparing and introducing a standard clot has proved to be useful and reproducible. Certain reservations exist in view of the fact that the clot is rapidly reduced to about 30 per cent of starting weight by washing in serum. It may be supposed that this effect is more or less a constant factor between experiments and we have shown that for a fresh clot a stable state is reached within 6 hours, after which changes in radioactivity in the blood and over the precordium may be considered to be due to the action of administered plasmin. Several reasons may be suggested for the varying results in clot lysis with plasmin. The plasmin preparation contains an activator of the dog's own plasminogen. Variations in the extent of this activation may play a part. The dosage or the method of administration or variation in the individual's susceptibility to the enzyme could also be responsible. The enzyme used has been uniformly active *in vitro*. The dose used, 0.5 ml./kg., probably represents a minimum effective level and was selected so as to disturb the clotting mechanism as little as possible. Incoagulability of the blood did usually appear, however, and in many instances the sutured wound would ooze during the maximum effect of the enzyme. It may prove that other methods of administration, such as giving the enzyme as a slow infusion, may result in better lysis with less clotting alterations. How great is the factor played by the individual's inhibitors of plasmin requires

further study. Although the plasmin inhibition test gives some promise of quantitating this aspect of the problem, considerably more experience with it is necessary before its usefulness is established. If it can be shown to represent accurately the individual's susceptibility to plasmin under a given set of circumstances it might be effectively applied to indicate the optimum dose required for clot lysis under the conditions present at the moment in question.

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DIURESIS IN HEMORRHAGIC HYPOTENSION*

EDWIN D. SAVION AND WILLIAM RICHISON

The effect of acute hemorrhagic hypotension on renal function in dogs has been studied by Corcoran and Page,¹ Selkurt,² and Phillips *et al*.³ Although direct measurement of blood flow from the renal vein during hypotension reveals a continuing, significant flow, oliguria or anuria supervenes at pressures between 60 and 100 mm Hg.² The exact mechanism of urine flow suppression is not clear. Compensatory renal vasoconstriction is present in varying degrees and would certainly seem to have some relationship to the anuria. However, denervation of the kidney or administration of the adrenergic 2-piperidinomethyl 11-benzodioxane (F933) has not increased urine flow during hypotension.³

One of us (W.R.) has observed an anuric patient without detectable peripheral blood pressure and unresponsive to norepinephrine who diuresed after administration of chlorpromazine. Recently Journeel⁴ in reporting on the use of chlorpromazine in the treatment of shock, mentioned that dogs bled to levels of 50 mm Hg excreted increased amounts of urine after treatment with the drug. The present report is an attempt to confirm these observations in dogs made hypotensive by hemorrhage.

METHOD

Eleven mongrel dogs weighing between 5.5 and 16.1 kg. were subjected to hemorrhagic hypotension by the method standardized by Wiggers.⁵ After induction of anesthesia using intravenous sodium pentobarbital 30 mg/kg. a polyethylene tube was threaded into the thoracic aorta by way of the

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femoral artery. A 3 way stopcock connected the tube with a mercury manometer or a Statham transducer for recording of blood pressure. Withdrawal of blood was accomplished through the 3 way stopcock with a 50 cc syringe. During each experiment an intravenous infusion of 5 per cent dextrose in water was maintained at 3 cc per minute.

Blood was withdrawn rapidly until the blood pressure reached 50 mm Hg and the pressure was maintained for 90 minutes at that level. Further bleeding to 30 mm was then done and the pressure sustained at this level for 45 minutes. The filtered blood was then reinfused by vein.

Urine was evacuated with an indwelling catheter in the bladder which was aspirated and irrigated with air every 15 minutes. Gentle supra pubic pressure was also used.

All animals were given 300 000 units of procaine penicillin at the beginning of the experiment.

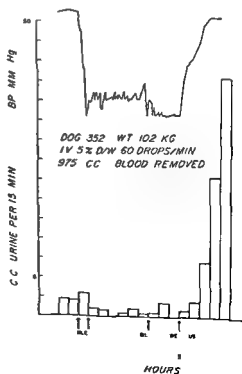
Chlorpromazine • 0.2 mg/kg was given intravenously to 7 dogs after the blood pressure had been 50 mm Hg for 45 minutes. Four of these dogs received a second similar dose of chlorpromazine after the blood pressure had been dropped to 30 mm Hg.

RESULTS

In the 4 dogs to which no drug was given there was suppression of urine flow at the hypotensive levels (Fig 1). Although complete anuria did not always occur, urine output was never more than 0.1 to 0.2 cc per minute.

After administration of chlorpromazine at 50 mm Hg there was an almost immediate increase in urine flow in 4 dogs with subsequent suppression on bleeding to 30 mm Hg (Fig 2). Urine flow rate at 50 mm was from 0.6 to 1.2 cc per minute.

Fig. 1 Oliguria at hypotensive levels in a typical control animal.



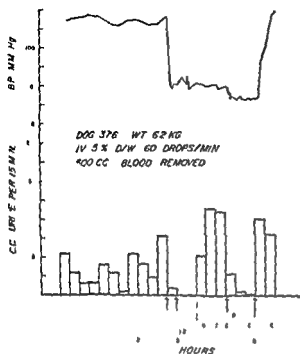


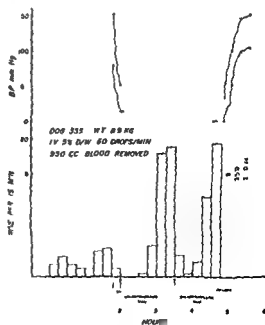
Fig 2 Increased urine flow at 50 mm Hg following administration of chlorpromazine 0.2 mg per kg. Further bleeding, to 30 mm Hg, caused recurrence of oliguria.

In 1 of these 4 dogs a second dose of chlorpromazine when the blood pressure had been 30 mm for 15 minutes with extreme oliguria resulted in a second period of diuresis (Fig 3). Flow at 30 mm was 1.3 cc per minute during the last collection period.

Two dogs showed no increase in urine flow after administration of the drug either at 50 mm or at 30 mm.

The remaining animal is of interest in that no immediate effect was noted after chlorpromazine was given. However, in an additional 15 minute period at 38-50 mm Hg was added to the experiment and during this time the dog excreted up to 1.3 cc per minute.

Fig 1 Increase in urine flow at 50 mm and at 30 mm Hg following successive doses of chlorpromazine 0.2 mg/kg.



Urine excreted during the hypotensive periods contained no protein specific gravity was 1.020 to 1.022 Sodium content was 3.7 mEq/L in 1 dog and was 29.0 mEq/L in another during diuresis at 50 mm

DISCUSSION

A number of possibilities may be proposed to explain the increased urine flow in the described circumstances.

1 Effect on posterior pituitary The secretion of antidiuretic hormone by the posterior pituitary in response to the stimulus of hemorrhage may have been blocked by chlorpromazine. Parrish and Levine⁷ recently reported a decrease in antidiuretic like substance in the urine of human beings following the drug.

2 Effect on kidney a) *On the renal cells* Some direct effect of the drug on the nephron may have either inhibited active water reabsorption in the distal tubules and collecting ducts or prevented proximal reabsorption of solutes. The concept of active water secretion in the renal tubules has been postulated in some quarters as a mechanism for urine dilution.⁸ From this premise it may well be asked if chlorpromazine might have promoted tubular water secretion. b) *On the blood vessels* An increase in glomerular capillary permeability allowing indiscriminate passage of large molecules into Bowman's capsule might result in increased fluid passage into the tubules due to lowering of the intravascular colloid osmotic pressure. This ultrafiltrate would no longer be protein free. The absence of proteinuria during the diuresis would seem to make this possibility less likely in the present situation.

By its known adrenolytic effects⁹ chlorpromazine may have inhibited renal vasoconstriction and lowered renal arteriolar resistance. Handley and Moyer¹⁰ injected dibenzylamine directly into one renal artery as an adrenergic blockade and demonstrated less depression of renal function in the treated kidney than in the opposite kidney during hemorrhagic hypotension in 2 of 3 dogs. Direct renal blood flow measurements in dogs by Brandfonbrener and Geller¹¹ revealed an increase in flow during hypotension following administration of dibenzamine. Alteration of renal hemodynamics by chlorpromazine in a similar manner could be a likely explanation for the present phenomenon and offers an avenue for further experimental exploration.

The fact that 2 of the 7 treated dogs did not diurese deserves further comment. It may first be pointed out that under the experimental conditions satisfactory urine output was not uniformly obtained during the preliminary normotensive periods. In addition it should be explained that even under conditions of maximal preliminary water diuresis the antidiuretic response stimulated by hemorrhage alone would probably mask minor degrees of diuresis induced by one or a combination of the aforementioned possible mechanisms. Other variables include the antidiuretic effect of pentobarbital and variations in the depth of anesthesia.

Another factor affecting the consistency of the results is the severe hypoproteinemia observed in all of the dogs. The extensive bleeding up to 106 cc/kg plus the dilution of remaining blood by the continuous infusion of glucose solution combined to produce plasma protein levels of 2.5 to 3.0 gm per cent. This reduction of plasma colloid osmotic pressure could be a factor in maintaining effective capillary filtration pressure. If one assumes that

renal arteriolar capillary pressure is approximately $\frac{2}{3}$ of aortic blood pressure and that plasma colloid osmotic pressure is roughly proportional to the protein concentration then the capillary filtration pressure will be 10 mm Hg when the blood pressure is 30 mm and the serum protein is 2.5 gm per cent. These assumptions disregard factors of arteriolar resistance and tissue pressure but serve to illustrate a means whereby variations of plasma protein may influence urine flow at hypotensive levels. It has been well demonstrated by Bojesen¹ in normotensive dogs that a minor depression in plasma protein without change in plasma volume can bring about a dilution diuresis.

Subsequent studies to be reported in detail indicate that these factors were instrumental in producing variation. It is of interest that although the control animals became equally as hypoproteinemic diuresis did not ensue. The present data give no inkling as to the exact role of chlorpromazine in producing diuresis but would certainly seem to confirm Fournel's impression.

The dose of chlorpromazine used in this experiment was considerably smaller than that usually given in studies of survival after shock in rats and dogs. We gave 2.0 mg/kg to a dog at 50 mm Hg with immediate bradycardia, profound drop in blood pressure, respiratory arrest and death in a matter of minutes. Survival studies using smaller doses of chlorpromazine would be of interest.

SUMMARY

Urine flow up to 1.5 cc per minute was observed in dogs bled to levels of 50 and 30 mm Hg aortic blood pressure. This occurred only after administration of a small dose of chlorpromazine.

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THE EXPERIMENTAL PRODUCTION OF A REVERSIBLE STATE OF CHRONIC PROGRESSIVE EDEMA*

JULIUS H. JACOBSON, II, JOHN PARACH AND FERDINAND F. McALLISTER

The study of the pathogenesis of the various states of chronic edema has been retarded by the lack of suitable experimental preparations. McKee Schilling, Tischkoff and Hyatt¹ have contributed to this problem by showing that ascites forms in the dog when the inferior thoracic vena cava is constricted. Such preparations, however, have been somewhat unpredictable regarding the duration of the ascites. Some animals have formed ascites slowly while others have died from over constriction. The method is not applicable when one wishes to use the dog as its own control after the ascites has been produced or where operation and anesthesia are undesirable in the experimental design.

In working out a method for the experimental constriction of larger blood vessels² with a pneumatic cuff it occurred to us that this technique might be used to produce easily controllable ascites.

METHOD

A pneumatic rubber cuff† is used to constrict the inferior thoracic vena cava of the dog. The cuff with its method of application is illustrated in Figures 1 and 2. It encircles the vena cava and is sewed together by means of

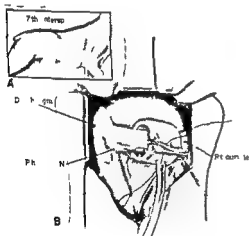
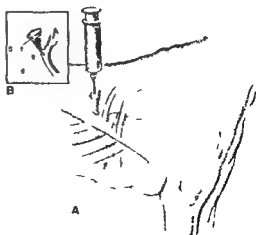


Fig. 1

Fig. 2 (A) Phantom drawing showing pneumatic cuff in place with needle being introduced subcutaneously into catheter tip. (B) Cross section of catheter tip.



Columbia University Medical Center

With the technical assistance of Mr. Gerard M. D'Alessio

†Made for us by the Davol Rubber Co. Providence R. I.

cloth tapes at either extremity of the balloon. Leading from the balloon is a rubber catheter that has at its end a self-sealing rubber diaphragm. This is led out of the chest and allowed to remain in the subcutaneous tissues after closure of the incision. Subsequently, when the animal has recovered from the operative procedure it is possible by injecting water into the self-sealing rubber diaphragm to produce degrees of traumatic sustained occlusion of the vessel. The obstruction may then be released in an equally atraumatic fashion by withdrawing the previously injected fluid.

There are a number of important steps that must be observed when using the cuff. It is essential to employ a cuff approximately twice the diameter of the vena cava. A cuff measuring 2 cm in diameter is satisfactory and provides enough leeway to prevent angulation of the vessel. The loose areolar tissue surrounding the vena cava should be dissected away for a distance only sufficiently large to pass the cuff around the vena cava. The optimum site for placement is immediately adjacent to the right auricle rather than near the diaphragm where constant movement is likely to compromise the vena cava. The catheter leading from the cuff should be led out through the thoracotomy wound and tunneled for a short distance under the serratus and pectoralis muscles. This prevents development of any pneumothorax that might occur around the catheter from an air leak in the skin closure. The funnel-shaped self-sealing rubber diaphragm should be embedded beneath the level of the platysma muscle so as to prevent ulceration of the overlying skin. The catheter tip is best left in the non-dependent position near the vertebral column so that the seroma that frequently forms in the first post-operative week is at a distance from it. Such placement helps prevent infection at the time of seroma aspiration and decreases the duration of seroma persistence.

The animal is allowed to recover from the cuff installation. Control studies are obtained without the clouding influence of the operative procedure, and then the inferior vena cava is constricted. Fluid is injected into the catheter tip to the point where the animal becomes unconscious, presumably due to a sudden decrease in cardiac output. Immediately, a small amount of fluid is withdrawn, i.e. 0.25 to 0.5 cc. The animal promptly returns to consciousness. The resultant degree of constriction is the maximum that is tolerable and ascitic fluid forms within 1 to 2 weeks. Attempts to regulate constriction at the operating table or by measurements of femoral venous pressure have been erratic and occasionally lethal.

RESULTS

Ascites has been produced in 9 dogs using the above method. In 5 of these a reversible preparation was desired. 4 were successful. In 3 of the 4 ascites was made to develop and regress twice in the same animal.

In Figure 3 pertinent metabolic data from a typical experiment are shown. The analytic methods have been previously reported.^{3,4} This dog on metabolic balance received a constant intake of 155 mEq of sodium daily and distilled water *ad lib*. Intake and output were recorded daily. The dog was weighed frequently in the fasting state.

It is interesting that upon partial occlusion of the thoracic vena cava sodium retention began immediately and the excretion of sodium was drastically reduced within the first 24 hours. Thereafter sodium excretion was

PRODUCTION OF REVERSIBLE EDEMATOUS STATE
IN A DOG ON CONSTANT NaCl INTAKE

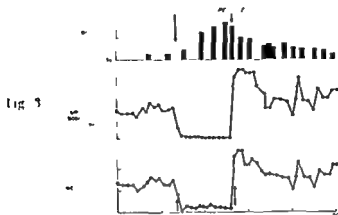


Fig 3

minimal to negligible—usually less than 25 mEq/day. Chloride excretion followed a similar pattern. This dog gained 12.5 kg in the 13 days in which the vena cava was occluded. He became markedly ascitic with edema noted in the hind legs. Upon release of the constriction diuresis of large amounts of edema fluid began immediately. In the ensuing 20 days the animal gradually returned to the control state.

SUMMARY

Utilizing a previously inserted pneumatic cuff placed around the inferior thoracic vena cava, ascites which is reversible has been produced atraumatically. Occlusion of the thoracic vena cava produces almost immediate sustained renal retention of sodium, a state which is perhaps analogous to a Chiari syndrome. Release of the obstruction produces prompt diuresis with immediate return of sodium excretion. The technique is being employed to study renal and hormonal factors in the pathogenesis of ascites. Studies relating to the role of the posterior pituitary in formation of ascites are being reported separately in this volume.

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Pre and Postoperative Problems in Water and Electrolyte Balance and in Nutrition

ELECTROLYTE BALANCES IN NEWBORN INFANTS FOLLOWING MAJOR SURGICAL PROCEDURES*

ELIZABETH COLETT AND LISA PAULSEN

A relationship between the metabolic changes following surgery and the release of adrenocortical hormones has been postulated by many investigators. Others have suggested that the adrenal gland plays a permissive role and that renal factors may be prominent in initiating the salt and water retention seen after operation.

Although the adrenal gland of the newborn infant is relatively larger than that of the adult, the major portion of it is composed of so called fetal cortical tissue which begins to undergo atrophy immediately after birth, leaving the infant with a small amount of adult cortical tissue composed of the zona glomerulosa and the zona fasciculata.¹ This anatomic event when correlated with such clinical and laboratory observations as the poor febrile response and failure of leukocytosis in infections, low blood sugar levels, high absolute eosinophil counts,²⁻⁴ absence of diurnal eosinophil variation⁵ and low levels of 17 hydroxycorticosteroids in the blood⁶ has suggested that the neonate is suffering from a relative hypoadrenism during the first days or weeks of life. Renal immaturity during this same period has been repeatedly demonstrated, tubular function being relatively less efficient than glomerular function.⁷

METHOD

1. Daily balances for sodium, potassium, chloride and nitrogen were performed on normal newborns and on newborns undergoing surgery. Adequacy of urine collections was insured by the use of indwelling catheters.

2. Blood chemistries were performed on capillary blood: a) sodium and potassium using the Beckman DU flame photometer; b) chloride by titration with mercuric nitrate using diphenylcarbazone as indicator; c) blood urea nitrogen using a micro modification of the Conway cup diffusion method.

3. Chemical analysis of urine and gastrointestinal tract losses were measured using the above techniques. Total nitrogens were measured by micro Kjeldahl. Osmolal concentrations of the urine were determined using the Fiske osmometer.

RESULTS

Normal newborns. Normal full term infants were given 5 per cent glucose or a multiple electrolyte solution (composition: sodium 25 mEq/l, chlo-

*Department of Pediatrics, University of Minnesota, Minneapolis, Minn. This work was supported by U. S. H. S. Grant No. H-7066.

ride 22 mEq/L and potassium 20 mEq/L) in amounts similar to those administered in the post operative period

While on glucose solution all the infants were in negative balance for sodium potassium chloride and nitrogen. The urinary excretions of electrolytes were remarkably constant averaging 1 to 5 mEq/kg/d. These figures are in agreement with published values in a similar study by Smith¹¹

When electrolyte solutions were offered balances immediately became positive and remained so throughout the period of study. The total electrolyte excretion remained about the same (4-5 mEq/kg/d)

Urine concentrations were highest during the period of limited water intake and fell when more water was provided. The highest concentration found in this period was 650 mOsm/L the usual range was 150 to 400 mOsm/L

Newborns following surgery. All infants undergoing surgery in the first week of life were followed on balance studies. In some cases it was possible to obtain collections for a pre operative period. The majority of the children were operated upon almost immediately following entry to the hospital and the data from these cases have been compared to that from the normal newborns

The total number of infants thus studied is 16. Of these 8 had operations for the relief of gastrointestinal obstruction, 5 for repair of tracheo esophageal fistula and 3 for repairs of sacral myelomeningoceles.

Anesthesia was cyclopropane or pentothal in all cases except for 2 of the neurosurgical procedures which were done under local anesthesia.

Operating times ranged from 15 min to 3 hr.

The group as a whole reveals certain constant features which were never observed in the normal newborn. In addition in these respects the post operative period in the newborn differs in several essential features from the post surgical period in the adult.

Three such studies are presented in detail as characteristic of the group as a whole.

Baby R. H. was born on 4/28 and admitted on 4/30. Weight at the time of admission was 3 kg. She was operated upon on the fourth day of life for repair of a tracheo esophageal fistula. Operating time was 3 hr and 25 min. Anesthesia was cyclopropane. During the 2 days prior to operation the child was in positive sodium and potassium balance. Urine output was 65 cc daily (within the range normally found in infants at this age) and the urine concentration was 350 mOsm/L on both days. Following surgery the child was in negative balance for sodium, potassium and chloride and nitrogen losses increased. Negative balances were maintained for 6 days following surgery. In addition the absolute amount of electrolytes excreted was greater than before surgery despite a lowered intake. Following surgery the urinary output increased and the water balance decreased. Associated with this was a decrease in urine concentration to a range of 200-250 mOsm/L which persisted throughout the period of study. During the post surgical period the serum sodium was 144 to 152 mEq/L.

Baby Cribb was admitted at the age of 54 hr and operated upon almost immediately. At surgery a massive meconium peritonitis was encountered. An ileostomy was performed. Operating time was 3 hr, anesthesia was cyclopropane and pentothal. No foreperiod collections were available. Following

surgery there was a tremendous output of sodium potassium and chloride. Nitrogen losses were moderately increased. These markedly negative electrolyte balances persisted to the time of the infant's death on the fourth post operative day. The urine output was high for an infant of this age and the urine output was greater than fluid intake. The urine solute concentration was never higher than 250 mOsm/l. The serum sodium concentrations were in the range of 115 to 150 mEq/L throughout the period of study.

Baby D.L. was operated upon at 21 hr. of age for low intestinal obstruction. Operating time was 2 hr. and 25 min. and anesthesia was pentothal. She weighed 3600 gm. at the time of admission. This infant not only excreted large amounts of salt in the urine, but did so in spite of large extrarenal losses incurred because of intestinal intubation. Her urine volume was high and the solute concentration following surgery was never greater than 270 mOsm/L. Her serum sodium values post surgically ranged from 140 to 160 mEq/L.

These cases represent the type of response seen. In every case there was negative balances for sodium potassium chloride and nitrogen in the post operative period. The losses of electrolytes were absolutely from 3 to 10 times that seen in normal infants observed during this same period. In addition urine volumes increased following surgery and the ratio of urine water to intake water was greater than in the newborn controls. The osmolar concentration of the urine remained low during this period. The magnitude of these changes correlated roughly with the nature of the surgical procedure, the length and type of anesthesia. The serum electrolyte concentrations were usually normal or elevated.

DISCUSSION

The post operative period in the adult has been characterized by a period of negative potassium and nitrogen balance, a variable degree of sodium and water retention, and a variable fall in the serum sodium concentrations.⁸⁻¹⁰ These changes occur during the period when excretion of 17OH in the urine is increased, although it has not always been possible to correlate the rise in compound F in the blood with the period of salt retention.¹⁰

The newborn infant subjected to surgery responds with an increase in potassium and nitrogen losses similar to that seen in the adult. However urinary losses of sodium have been found to increase in those cases in which it was possible to compare them with a pre surgical specimen, and to be greatly in excess of those seen in normal newborns on a comparable fluid and electrolyte intake. In addition urine output is regularly increased and urinary concentrations are low. The serum electrolytes are either normal or (more often) increased.

It is known that the infant has diminished renal function in the early days of life. However normal infants will concentrate to 600 mOsm/L¹¹ and the majority of our normal infants have shown concentrations as high as 400 mOsm/L. The infant following surgery seems unable to concentrate much higher than 200 to 250 mOsm/L—and this inability to concentrate may remain for many days despite the fact that the child is actually in negative water balance. It would seem that the water diuresis seen in the infant may well be the direct result of impairment of kidney tubular function following surgery.

Whether the increased electrolyte excretion observed following surgery is a consequence of the primary defect in water metabolism cannot be determined from the data at hand. The role of the adrenal in response to stress has been emphasized by many although there are others who hold that the adrenal has only a permissive function. If the sodium retention seen in the adult is in some way correlated with an increased secretion of adrenocortical hormones the following explanations for the failure of the infants to show this response may be offered: (1) The neonate suffers from hypoadrenalism. The evidence of Klein documenting a rise in plasma compound F levels in response to exogenous ACTH and to surgical stress would mitigate this possibility.¹² (2) The immature renal tubule is incapable of responding to a salt retaining hormone. (3) The newborn adrenal produces a salt losing hormone in response to ACTH. Klein has reported the presence of such hormone in young infants after stimulation with exogenous ACTH.¹³ (4) The changes seen are due to a pre-existing hormonal imbalance present in the newborn. Antagonism between DOC and other adrenocortical hormones have been reported in patients with Cushing's disease¹⁴ and in patients with adrenal virilism.¹⁵ The newborn infant pre-treated during the last trimester with high doses of maternal corticoids may react to the normal adrenal secretions called forth by surgical stress in an unexpected manner due to a withdrawal state.

SUMMARY

1. Balance studies on newborn infants and on newborns undergoing surgery are presented.
2. The infants subjected to surgery are in negative potassium and nitrogen balance during the post operative period in a manner similar to that seen in adults.
3. The infant post surgically tends to lose sodium and to have a water diuresis associated with low solute concentrations during this period in contrast to the salt and water retention seen in the adult.
4. Concentrations of electrolytes in the blood are normal or increased during this period.

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OSMOTIC DIURESIS AS TREATMENT IN SEVERE HYPONATREMIA*

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With the expanding scope of modern surgery has come an increase in the frequency with which major operative procedures are performed upon patients suffering from chronic depleting illnesses. These patients manifesting preoperatively varying degrees of fluid and electrolyte imbalance are often prone to develop severe hyponatremia after operation and are also more sensitive to the consequences of such hyponatremia. Severe liver disease and chronic congestive heart failure are the most frequent systemic disorders associated with lowered serum sodium concentrations postoperatively, although the chronic stimulation which accompanies certain lesions requiring operative therapy is of equal significance in this regard.

The primary genesis of postoperative hyponatremia in such patients is water loading, not salt loss, as an increased impetus to renal sodium conservation is a common feature of this syndrome. Impaired excretion of water loads occurs during the early postoperative period presumably as the result of increased antidiuretic hormone secretion by the neurohypophysis. After the second or third postoperative day the occurrence of continued water retention is most probably related to inadequate solute output as the low urine volumes then are characterized by solute concentrations well below the levels produced by the earlier ADH activity. A less negative nitrogen balance and continuing sodium retention now deprive the urine of its two primary solute components—urea and sodium plus accompanying anions. Under these circumstances a cumulative positive water balance of several liters may develop insidiously at the rate of only 300 to 400 ml/d. The result is marked hyponatremia with the attendant clinical manifestations as described so well by Wynn¹ and others.²

Low serum sodium concentrations may be restored to normal either by the

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addition of sodium (as hypertonic saline infusion) or by decreasing body water. Since patients in whom severe post-operative hyponatremia most frequently occurs are usually lacking in cardiopulmonary reserve, hypertonic saline is contraindicated because further extracellular fluid expansion may precipitate severe pulmonary edema. Decreasing body water by marked restriction of water intake may produce elevations of the serum sodium concentration, however the rise occurs too slowly to be therapeutically useful. A prompt decrease in body water resulting thereby in elevation of serum sodium concentration would be achieved if marked increases in urine flow could be produced by a diuretic agent which did not effect a saluresis. These preliminary studies were undertaken to ascertain if mannitol, a substance commonly used in renal physiology to promote osmotic diuresis, might be such an agent. Studies of the effect of mercurial diuretics in similar conditions are included for comparative purposes.

METHOD

Eight studies were performed on 5 patients, 3 of whom developed hyponatremia post-operatively and 2 in whom diuretic therapy was being carried out during evaluation for cardiac valvular surgery. One patient in this latter group was eunatremic but had not responded to other diuretic measures. Hypertonic saline therapy was considered inadvisable in all instances of hyponatremia.

Twenty five per cent solutions of mannitol* were administered by intravenous infusion at rates not exceeding 150 ml/hr. Mercurials were given subcutaneously in customary dosage.

Urine specimens were obtained by indwelling catheter in 4 cases and by voiding in 1 case.

Sodium and potassium concentrations in serum and urine were determined by flame photometry. Osmolality of serum and urine was measured with a freezing point depression osmometer.

RESULTS

In 4 of 6 instances a significant rise in serum Na concentration occurred following mannitol induced diuresis (See Table 1). However in the other 2 cases oral fluid intake continued to exceed water loss so that the overall fluid balance was unaltered by the mannitol diuresis. Two cases having mercurial diuresis show either a drop or no change in serum Na concentration.

Of interest also is the fall in serum K occurring in those cases showing elevations of serum Na.

Although comparable increases in urine flow resulted from both osmotic and mercurial diuresis, the contrasting changes in sodium excretion are striking (Table 2). While mannitol did indeed produce some increase in sodium excretion in 5 of 6 instances, this higher rate of sodium loss is still inconsequential with respect to any effect on serum Na concentration or body sodium content.

DISCUSSION

These data show that in the presence of a marked tendency to urinary Na conservation, the administration of a nonelectrolyte osmotic diuretic will in

*Merck Sharpe and Dohme supplied the mannitol used.

Table 1 Effect of diuresis on serum osmolality and electrolytes

PATIENT DIURETIC AGENT	BEFORE DIURESIS			AFTER DIURESIS		
	SERUM NA mEq/L	SERUM K mEq/L	SERUM OSMOLALITY mOsm/L	SERUM NA mEq/L	SERUM K mEq/L	SERUM OSMOLALITY mOsm/L
SE						
Mannitol	125	4.2	260	129	4.4	264
AL I						
Mannitol	117	6.4	250	124	5.4	260
AL II						
Mannitol	125	4.8	261	130	4.5	267
BS						
Mannitol	118	4.1	278	138	3.3	332
WR						
Mannitol	134	5.1	278	135	5.4	280
KO						
Mannitol	123	5.0	266	118	6.0	273
SE						
Hg	130	4.8	265	126	3.7	261
WR						
Hg	134	4.7	278	135	4.5	269

crease urine output without significantly altering sodium homeostasis. Under clinically appropriate circumstances as delineated previously, this effect is useful in the treatment of symptomatic hyponatremia resulting from water excesses.

To produce a rise in serum Na concentration by osmotic diuresis, calculations of the needed amount of mannitol first require estimation of the extent of negative water balance necessary. It is essential that the intake component of this estimation include the volume of mannitol solution infused.

Table 2 Effect of diuresis on water and sodium excretion

PATIENT DIURETIC AGENT	BEFORE DIURESIS		DURING DIURESIS	
	URINE FLOW ml/min	NA EXCRETION μ eq/min	URINE FLOW ml/min	NA EXCRETION μ eq/min
SF				
Mannitol	0.40	2.20	1.76	6.31
AL I				
Mannitol	0.66	0.78	2.29	0.57
AL II				
Mannitol	0.18	0.30	1.88	1.20
BS				
Mannitol	0.38	1.10	2.18	1.88
WR				
Mannitol	0.28	3.10	1.58	18.2
KO				
Mannitol	1.08	1.60	1.98	1.67
SE				
Hg	0.36	1.75	2.14	18.7
WR				
Hg	0.80	6.01	1.79	37.2

Failure to do so may prevent an increase in serum Na level even though diuresis ensues (See Cases W R and K O)

After estimating the desired water loss this is converted to a urine flow rate (ml/min) by relating it to the period of time over which the serum Na change is to occur. After determining pre-diuretic urine flow (V) urine osmolality (U) and plasma osmolality (P) the number of milliosmols of osmotic diuretic required is then calculated in the following manner

$$\frac{UV}{P} = C_{osm} \text{ (pre-diuretic osmolar clearance)} \quad (1)$$

Since at constant levels of ADH activity U and P remain constant substitution of the desired value V' for V gives

$$\frac{UV'}{P} = C_{osm} \text{ (osmolar clearance of diuresis)} \quad (2)$$

C_{osm} may result from either an increase in V ($V \rightarrow V'$) or in U ($U \rightarrow U'$) therefore (2) may be rewritten

$$\frac{U'V}{P} = C_{osm} \quad (3)$$

$$\text{or } U = \frac{P \times C'_{osm}}{V} \quad (4)$$

The requisite increase in milliosmolar excretion (1) is then

$$U - U = 1 \text{ mOsm (needed for } V \rightarrow V') \quad (5)$$

25 per cent mannitol contains approximately 1400 mOsm/L and thus

$$\frac{1 \text{ mOsm}}{1400} = \text{liters mannitol to be given} \quad (6)$$

This is given intravenously at the rate of approximately 100 ml/hr so that dilution of mannitol in extracellular fluid and renal excretion will both occur at a rate sufficient to prevent extracellular fluid expansion with resultant pulmonary edema

Theoretical results obtained by fitting the data from these studies into the above calculations agree well with the observed changes within the range of experimental error. And in further therapeutic trials in which the calculations have been carried out prior to mannitol administration the anticipated results have been observed.

SUMMARY

Osmotic diuresis induced with mannitol has been shown to produce salt poor urine in clinical states in which renal sodium conservation is marked. This effect is useful in the treatment of dangerous postoperative dilutional hyponatremia.

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FURTHER STUDIES IN BODY FLUID METABOLISM II THE RELATIVE EFFICACY IN MAN OF SODIUM BICARBONATE AND SODIUM LACTATE AS ALKALIZING COMPOUNDS, AND OF AMMONIUM CHLORIDE AND HYDROCHLORIC ACID AS ACIDIFYING COMPOUNDS*

JAMES D HARDY, JOHN O DAVIEER JR AND M DON TURNER

Commercially prepared sodium lactate has long been the agent principally used intravenously to combat acidosis in clinical practice Yet sodium bicarbonate would seem to have certain advantages over lactate and why the former has not been more frequently employed for this purpose has not been entirely clear One prominent reason has been a common impression that a sodium bicarbonate solution could not be autoclaved without producing chemical alterations which rendered it unfit for intravenous use Notwithstanding since the purpose in using either compound in acidosis is to make available additional fixed base with as little other effect as possible sodium bicarbonate would appear to be a more satisfactory substance the CO_2 introduced is readily excreted by the lungs whereas infused lactate ion must be metabolized Thus the first part of this study consisted of a clinical comparison of sodium bicarbonate with sodium lactate

The second portion of the study consisted of a clinical comparison of HCl with NH_4Cl as acidifying agents Despite the fact that in the literature it has frequently been stated that hydrochloric acid may also be used it has been difficult to find physicians who did not prefer to employ ammonium chloride in the treatment of alkalosis—in fact few indeed appear to have used hydrochloric acid intravenously for this purpose Nevertheless the infusion of NH_4Cl does at times produce marked visual and gastrointestinal disturbances and in the presence of liver failure the infusion of additional ammonium ion may be hazardous In the present study the infusion of HCl in sodium chloride solution caused no symptoms and the infusion of NH_4Cl at the rate employed here occasioned no particular discomfort

METHOD

In the case of each of the four substances—sodium bicarbonate sodium lactate hydrochloric acid and ammonium chloride—167 mEq/1000 cc were infused at a virtually constant rate over a period of $1\frac{1}{2}$ hours Blood samples were drawn before infusion after $\frac{1}{2}$ hour after 1 hour and $\frac{1}{2}$ hour after the end of the infusion Measurements of blood pH (Beckman pH meter) and carbon dioxide content (Van Slyke manometric apparatus) were employed to calculate carbon dioxide combining power Plasma chloride levels were determined but despite individual changes the averaged experimental values for the groups did not vary significantly from the control levels

*From the Department of Surgery University of Mississippi Medical Center Jackson Mississippi This work was performed under Army Contract No DA 49 007 MD 627 Office of the Surgeon General Department of the Army
With the technical assistance of Virginia Ward and Thelma Carter

RESULTS

Four groups of 10 patients each were studied. One group received sodium lactate, one sodium bicarbonate, one hydrochloric acid, and one ammonium chloride. Except for an occasional minor aberration, the subjects were in normal acid base balance.

A representative set of data from each group is shown in Table 1. As was to be expected, the precise degree of change in any of the several values in a given individual varied considerably, but by averaging the changes for all patients it was found that sodium bicarbonate and sodium lactate were equally effective as alkalinizing agents, and that hydrochloric acid and ammonium chloride were equally effective as acidifying agents. The infusion of NaHCO_3 and sodium lactate resulted in a rise in blood pH, plasma carbon dioxide combining power, and total carbon dioxide content. The infusion of NH_4Cl and HCl produced a fall in blood pH, a fall in plasma carbon dioxide combining power, and a fall in total carbon dioxide content of blood. The plasma chloride level rose following the infusion of both NH_4Cl and HCl , but as anticipated, there was no consistent change in the chloride level following the infusion of sodium lactate and sodium bicarbonate.

All alterations in the measured values usually were progressive from the beginning of the infusion, and had not returned to normal $\frac{1}{2}$ hour after the end of infusion.

Table 1. Chemical Changes in Venous Blood from a Representative Patient in Each Group

SOLUTION INFUSED	NAME	SAMPLE	pH	CO CONTENT VOL. 90	CO CAPACITY mEq/L	Cl
NaHCO_3	D.S.	C	7.45	61.83	29.8	104.5
		1	7.50	62.04	30.0	106.2
		2	7.55	63.29	32	106.2
		3	7.57	69.87	35	99.6
Na Lactate	C.S.	C	7.33	53.56	25	100.0
		1	7.49	58.49	31	101.6
		2	7.44	61.21	32	108.4
		3	7.51	61.08	31	100.0
HCl	M.I.	C	7.40	60.83	24	103.6
		1	7.35	55.90	23.6	112.3
		2	7.37	50.97	21	111.6
		3	7.37	47.68	21	108.7
NH_4Cl	I.W.	C	7.40	58.25	28	108.2
		1	7.35	53.56	27	112.2
		2	7.32	45.56	24	116.5
		3	7.31	45.50	24	112.2

FURTHER STUDIES IN BODY FLUID METABOLISM II THE RELATIVE EFFICACY IN MAN OF SODIUM BICARBONATE AND SODIUM LACTATE AS ALKALIZING COMPOUNDS AND OF AMMONIUM CHLORIDE AND HYDROCHLORIC ACID AS ACIDIFYING COMPOUNDS*

JAMES D. HARDY, JOHN O. DAMPEER, JR. AND M. DON TURNER

Commercially prepared sodium lactate has long been the agent principally used intravenously to combat acidosis in clinical practice. Yet sodium bicarbonate would seem to have certain advantages over lactate and why the former has not been more frequently employed for this purpose has not been entirely clear. One prominent reason has been a common impression that a sodium bicarbonate solution could not be autoclaved without producing chemical alterations which rendered it unfit for intravenous use. Notwithstanding, since the purpose in using either compound in acidosis is to make available additional fixed base with as little other effect as possible, sodium bicarbonate would appear to be a more satisfactory substance: the CO_2 introduced is readily excreted by the lungs, whereas infused lactate ion must be metabolized. Thus, the first part of this study consisted of a clinical comparison of sodium bicarbonate with sodium lactate.

The second portion of the study consisted of a clinical comparison of HCl with NH_4Cl as acidifying agents. Despite the fact that in the literature it has frequently been stated that hydrochloric acid may also be used, it has been difficult to find physicians who did not prefer to employ ammonium chloride in the treatment of alkalosis—in fact, few indeed appear to have used hydrochloric acid intravenously for this purpose. Nevertheless, the infusion of NH_4Cl does at times produce marked visual and gastrointestinal disturbances, and in the presence of liver failure the infusion of additional ammonium ion may be hazardous. In the present study, the infusion of HCl in sodium chloride solution caused no symptoms, and the infusion of NH_4Cl at the rate employed here occasioned no particular discomfort.

METHOD

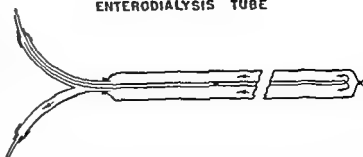
In the case of each of the four substances—sodium bicarbonate, sodium lactate, hydrochloric acid, and ammonium chloride—167 mEq/1000 cc were infused at a virtually constant rate over a period of $1\frac{1}{2}$ hours. Blood samples were drawn before infusion, after $\frac{1}{2}$ hour, after 1 hour, and $\frac{1}{2}$ hour after the end of the infusion. Measurements of blood pH (Beckman pH meter) and carbon dioxide content (Van Slyke manometric apparatus) were employed to calculate carbon dioxide combining power. Plasma chloride levels were determined, but despite individual changes, the averaged experimental values for the groups did not vary significantly from the control levels.

*From the Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi. This work was performed under Army Contract No. DA-49-007-MD-627, Office of the Surgeon General, Department of the Army.

With the technical assistance of Virginia Ward and Thelma Carter.

ENTERODIALYSIS TUBE

Fig 1 Fluid is perfused through the central plastic tube to the distal end of the cellophane tubing and back through the entire length of cellophane tubing to be collected at the outflow tube shown



well as that shown for introduction into the stomach or jejunostomy. Using an automatic infusion-extraction apparatus with a program timer, it is possible to fill and empty the tube to collect samples, and to interrupt the infusion time at regular intervals for periods up to 24 hours.

Thirty female dogs weighing 10 to 12 kg were used for the experimental preparations which consisted of left nephrectomy and either a Maydl jejunostomy or an isolated intestinal loop (Thiry Vella) fistula varying in length from 3 to 7 feet. The second stage (right nephrectomy) procedure was performed through a flank incision about two weeks after the first operation when the dogs had regained normal health and weight. Within 4 days the cellophane tube was introduced into the enteric fistula and perfusion of the lumen of the cellophane tube was begun using a perfusate free from K and N having the following composition: Na 135 mEq/L, Ca 5 mEq/L, Mg 2 mEq/L, Cl 110 mEq/L, HCO_3^- 15 mEq/L, lactate 17 mEq/L and dextrose 0-10 gm/L. The above amounts and flow rates were varied in accordance with specific experimental objectives. Diet usually consisted of 25 per cent glucose solution. Samples of the perfusate were taken from the cellophane tube at appropriate intervals and were analyzed for Na, K, Ca, Mg, Cl, HCO_3^- , P, urea, NPN, creatinine, glucose, pH and D.O. N^{15} , P^{32} and K^{42} by standard methods with occasional minor modifications. Blood was analyzed for the same constituents. Deuterium oxide (heavy water), radio-sodium²⁴, radiopotassium⁴² and radiophosphorus³² were administered intravenously and their rates of appearance were measured in the cellophane tubing. In additional experiments these isotopes were introduced into the cellophane tube and the appearance rates in the blood were determined. The kinetics of transfer of these respective substances in both directions between the blood and the lumen of the cellophane tube within the intestine were thereby evaluated.

From the measured changes in concentration of the various substances in the dialysates and from the volume collected the total amounts of the several measured constituents removed or absorbed were calculated.

RESULTS

After determining the *in vitro* permeability characteristics of the cellophane to potassium and urea, a closed cellophane tube was placed in the stomach of a normal human and the appearance half times of potassium and nitrogen were found to be about one hour with a similar finding when the cellophane tube was placed in the dog jejunostomy. Instillation of D.O., Na^{24} and K^{42} directly into the small intestine with serial blood measurement showed an absorption half time of ten minutes while introduction of these

Effect of Heat upon Sodium Bicarbonate Solution No significant change was detected in sodium bicarbonate solution upon autoclaving

DISCUSSION

It is clear from these studies that intravenous sodium bicarbonate and sodium lactate solutions are approximately equally effective as alkalinizing agents. It is also clear that hydrochloric acid and ammonium chloride are both effective acidifying agents. To avoid pain at the site of infusion the HCl solution must be rendered isotonic with additional solute (here NaCl) and to avoid distressing symptoms of nausea vomiting and visual disturbances the ammonium chloride must not be administered too rapidly.

SUMMARY

1 Autoclaving sodium bicarbonate solution produced no chemical alterations that could be detected.

2 Sodium bicarbonate and sodium lactate proved equally effective alkalinizing compounds. Presumably therefore the more rapidly disposable anion of the former should render it superior to the latter for use in some patients in critical circumstances.

3 Hydrochloric acid and ammonium chloride were almost equally effective acidifying agents. When rendered isotonic by the addition of NaCl the HCl solution does not cause pain at the site of infusion. In the presence of borderline hepatic function HCl would appear to possess certain theoretical advantages over NH_4Cl .

ENTERODIALYSIS: AN APPROACH TO THE PROBLEM OF RENAL SUBSTITUTION*

PAUL R. SCHLOERB

In an attempt to combine the relative merits of intestinal perfusion and extracorporeal hemodialysis for removal of accumulated toxic metabolites in uremia a method using perfusion of a closed cellophane tube within the intestinal tract is being developed. An interim evaluation of this method in the normal human, nephrectomized dogs and one terminally uremic patient is described in this preliminary report.

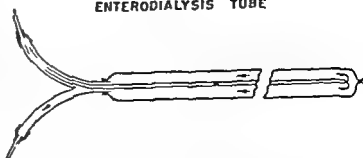
METHOD

A closed cellophane tube has been fashioned in various ways including that shown in Figure 1. Appropriate modifications have been employed to permit through and through perfusion within an isolated intestinal loop as

*From the Department of Surgery, University of Kansas School of Medicine, Kansas City, Kansas, and the Kansas City Veterans Administration Hospital, Kansas City, Missouri. Supported in part by the Surgery Development Fund. Presently supported by U.S.P.H.S. Grant #H 2363.

ENTERODIALYSIS TUBE

Fig 1 Fluid is perfused through the central plastic tube to the distal end of the cellophane tubing and back through the entire length of cellophane tubing to be collected at the outflow tube shown



well as that shown for introduction into the stomach or jejunostomy. Using an automatic infusion-extraction apparatus with a program timer it is possible to fill and empty the tube to collect samples and to interrupt the infusion time at regular intervals for periods up to 24 hours.

Thirty female dogs weighing 10 to 12 kg were used for the experimental preparations which consisted of left nephrectomy and either a Maydl jejunostomy or an isolated intestinal loop (Thiry Vella) fistula varying in length from 3 to 7 feet. The second stage (right nephrectomy) procedure was performed through a flank incision about two weeks after the first operation when the dogs had regained normal health and weight. Within 4 days the cellophane tube was introduced into the enteric fistula and perfusion of the lumen of the cellophane tube was begun using a perfusate free from K and N having the following composition: Na, 135 mEq/L; Ca, 5 mEq/L; Mg, 2 mEq/L; Cl, 110 mEq/L; HCO_3^- , 15 mEq/L; lactate, 17 mEq/L; and dextrose, 0-10 gm/L. The above amounts and flow rates were varied in accordance with specific experimental objectives. Diet usually consisted of 25 per cent glucose solution. Samples of the perfusate were taken from the cellophane tube at appropriate intervals and were analyzed for Na, K, Cl, Mg, HCO_3^- , P, urea, NPN, creatinine, glucose, pH, and D_2O . Na^{22} , P^{32} , and K^{42} by standard methods with occasional minor modifications. Blood was analyzed for the same constituents. Deuterium oxide (heavy water), radio-sodium²², radiopotassium⁴², and radiophosphorus³² were administered intravenously and their rates of appearance were measured in the cellophane tubing. In additional experiments these isotopes were introduced into the cellophane tube and the appearance rates in the blood were determined. The kinetics of transfer of these respective substances in both directions between the blood and the lumen of the cellophane tube within the intestine were thereby evaluated.

From the measured changes in concentration of the various substances in the dialysates and from the volume collected the total amounts of the several measured constituents removed or absorbed were calculated.

RESULTS

After determining the *in vitro* permeability characteristics of the cellophane to potassium and urea, a closed cellophane tube was placed in the stomach of a normal human and the appearance half times of potassium and nitrogen were found to be about one hour with a similar finding when the cellophane tube was placed in the dog jejunostomy. Instillation of D_2O , Na^{22} , and K^{42} directly into the small intestine with serial blood measurement showed an absorption half time of ten minutes while introduction of these

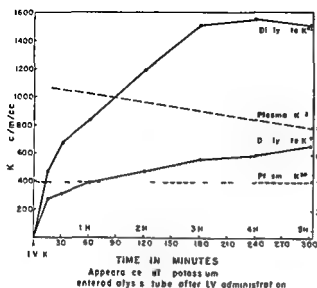


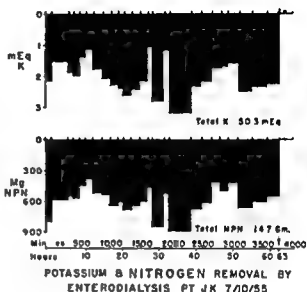
Fig 2 Appearance rates of radio active and stable potassium in the cellophane tubing are shown. Volume measurements indicate that the higher concentrations of K^{42} and K^{40} in the tubing are not the result of water loss but reflect the normally increased concentration of potassium in the intestinal lumen.

isotopes into the closed cellophane tube within the intestine showed an absorption half time in the blood of about one hour. After intravenous administration of these isotopes (Fig 2) serial measurement of their appearance rates into a potassium and nitrogen free perfusate solution contained in the cellophane tube within the intestine showed an appearance half time of about $1\frac{1}{2}$ hours indicating that the K , N , and H_2O in the cellophane were in effect removed indirectly from the blood and not merely from the intestinal contents.

Metabolic balance data on normal and bilaterally nephrectomized dogs during enterodialysis indicated removal of potassium and nitrogen without marked change in sodium, Ca , Mg , Cl , HCO_3 , or P . The rates of removal in uremia of potassium with cellophane tubing was 0.2–0.5 mEq/ft/hr while the non protein nitrogen removal rate was 63 to 103 mg/ft/hr. The maximum survival time of nephrectomized dogs without additional supportive treatment has been 11 days. Untreated controls survived 2 to 5 days.

One uremic patient, a 46 year old man with arteriolonephrosclerosis and malignant hypertension, was terminal and becoming moribund despite the usual medical supportive measures and was treated by enterodialysis using 7 feet of $2\frac{1}{2}$ " diameter cellophane introduced into the small intestine by jejunostomy under local anesthesia. Perfusion was begun (Fig 3) using an average volume of perfusate of 400 ml which was allowed to remain in the tube for an average of 150 minutes. This volume was alternately infused and aspirated for 63 hours when he expired suddenly from what proved at autopsy to be a large myocardial infarction. During this period 50 mEq of potassium and 147 gm of non protein nitrogen were removed without significant change in other electrolytes. Significant change in serum electrolyte and NPN values was not observed although these latter data are not adequate for positive interpretation. Six hours after initiating enterodialysis reorientation and arousal from coma occurred. Of interest was the observation that at the shorter perfusion time (60 to 75 minutes) the removal of potassium and nitrogen were double the overall average corresponding to daily rates of removal of 36 mEq of potassium and 12 gm of nitrogen.

Fig 3 The width of each block represents the time during which the 400 ml of perfusate was allowed to remain in the cellophane tube. The solution was then aspirated completely and the tubing was refilled with another 400 ml of K and NPN free solution. Removal rate = mEq or mg/time. Rate is greater at the shorter times.



DISCUSSION

The formidable analytical problem of attempting to identify all or most of the accumulated metabolites in uremia makes it necessary to select certain entities which correlate with the severity of renal insufficiency. Potassium, non-protein nitrogen, urea, and creatinine have been selected for study in these experiments with the realization that alterations in these substances may only indirectly reflect many other metabolic abnormalities.

A relatively rapid exchange and transfer of potassium, sodium, and water from the blood to the lumen of the cellophane tube within the intestine has been demonstrated by isotopic and chemical methods. Because of a similar appearance rate for non-protein nitrogen, urea, and creatinine in the cellophane and absence of a decrease in this rate of appearance over a prolonged period, it is assumed that a comparable transfer rate of NPN from blood to intestinal lumen exists. Experimental evaluation of this assumption is in progress.

The transfer or exchange rate of substances across the cellophane membrane within the intestine depends upon several factors including concentration gradient across the membrane, temperature, enteric pressure, normal function of the intestinal epithelium, presence or absence of intestinal obstruction (kinking or intussusception), and mesenteric blood flow. The amount of substances exchanged or transported across the membrane is dependent upon both the exchange rate and the surface area. Since the cellophane tubing diameter is constant for any given size of cellophane, surface area may be expressed most simply as a function of length. It is clear, therefore, that the amounts of metabolites removed are proportional to the length, diameter, and contained volume of the cellophane tube in addition to the factors previously mentioned.

Problems encountered have included occurrence of intussusception in 30 per cent of the animals but not in the patient. Development of anemia has been a limiting factor in survival studies and adequate dietary maintenance has been hindered by the uniform occurrence of vomiting while jejunal tube feeding has produced diarrhea.

The metabolic balance data on the single uremic patient studied appears to indicate that amounts of potassium and nitrogen may be removed selectively in amounts in excess of the daily dietary requirement but present laboratory experience must be extended before clinical application can be considered in other than terminal renal failure due to irreversible kidney disease.

SUMMARY

A new method using perfusion of cellophane tubing within the intestine for selective removal of toxic metabolites in uremia is being developed. The dynamics of exchange between the blood and the lumen of the cellophane tube are presented. Maximum selective daily removal of 36 ml q of potassium and 12 gm of non protein nitrogen was accomplished in one terminally uremic patient by this method.

OBSERVATIONS ON THE ELECTROLYTE AND OSMOLAL CONCENTRATION CHANGES OF SERUM AND URINE IN RESPONSE TO VARIABLE CONCENTRATIONS OF INTRAVENOUS SODIUM CHLORIDE, GLUCOSE AND WATER IN NORMAL SUBJECTS*

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AND JAMES O. ROBINSON

The response of the human kidney to the administration of parenteral fluids possesses great variability. Particularly is this true when the renal excretion of intravenously administered water and sodium salts by the normal unstressed individual is compared to that observed in patients undergoing surgical operations. The continuous oral or intravenous infusion of 5 per cent glucose and water consistently produces a negative water balance in the normal person.¹ Under such conditions the urine volume may exceed the amount of infused when as little as 100 ml/hr is given continuously for 24 hours. Various explanations have been suggested to account for this phenomenon but none appear adequate, for it is probable that many factors are responsible.^{2,3,4} During operation and in the immediate postoperative period however the ability of the kidney to excrete comparable loads of water and salt may be sharply depressed.^{4,5,6} The overenthusiastic administration of both water and sodium salts during this period invites serious and even lethal postoperative complications.

In the absence of significant extrarenal loss of body electrolyte (e.g. gastrointestinal suction, intestinal fistulae, burns, severe infections) the adminis-

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itation of carbohydrate and water solutions for the provision of water needs during operation and in the immediate postoperative period has achieved wide acceptance. Certain investigators however feel that a more physiological approach could be accomplished by the administration of hypotonic balanced electrolyte solutions during periods of maximum operative and postoperative stress.

This report details initial studies done on normal human volunteers, further investigating the variable parameters of induced glucose and water diuresis and the effect upon this diuresis of hypotonic amounts of sodium chloride.

METHOD

Seven normal volunteers were used in this study. Because of space limitations only the results obtained in 6 studies of 2 members of the resident surgical staff will be presented.

Without prior dietary control all subjects were fasted overnight. A base line of rate of urine secretion and concentration was established by a specimen obtained during the 2 hours prior to starting the infusion. The infusion period was 10 hours. Subjects lay quietly in bed and stood erect only to void. Hourly urine specimens were collected. Venous blood samples were drawn at 0, 3, 7 and 10 hours. Fasting continued for 14 hours following completion of the infusion. Urine excreted during this period was collected and examined with the other specimens.

With subjects serving as their own controls the infusions were repeated at periods varying from 3 to 7 days under comparable circumstances of environment and room temperature. Relative humidity was not controlled. The solutions used were 5 per cent glucose in water, 0.15 per cent NaCl in 5 per cent glucose and 0.30 per cent NaCl in 5 per cent glucose solution. Rates of administration were controlled by a constant infusion pump and varied from 204 to 264 ml/hr for the 10 hour period.

Renal function was determined by 24 hour creatinine clearance test. Sodium and potassium concentrations of serum and urine were determined by flame photometry. Chloride was done by a modification of the Volhard procedure. Serum proteins were determined by the Wolfson modification of the Weichselbaum technique.⁹ Voided urine specimens were frozen immediately and subsequent osmotic pressure determinations done with the Fiske Osmometer. Serum osmolality was similarly determined.

RESULTS

Urine volume and excretion rates. Ignoring insensible loss, subject Al was in negative water balance at the end of 24 hours regardless of the type of solution given (Fig. 1). His largest negative balance was achieved with 0.30 per cent NaCl/glucose infusion. Subject Ro had the largest negative balance of all subjects studied — 810 ml at the end of 24 hours when given glucose alone (Fig. 2). He demonstrated a positive balance of 98 ml with 0.15 per cent NaCl/glucose and 373 ml with 0.30 per cent NaCl/glucose at the end of 24 hours. If the insensible loss is estimated as 40 ml/hr, it is evident that all studies ended in a net negative water balance regardless of the type of infusion given. Maximum excretion rate was 7 ml/min. This was observed during administration of glucose alone.

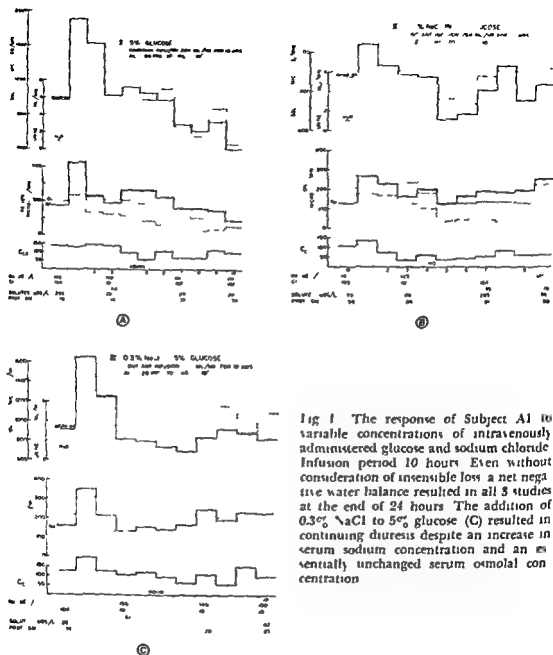


Fig 1 The response of Subject A1 to variable concentrations of intravenously administered glucose and sodium chloride. Infusion period 10 hours. Even without consideration of insensible loss a net negative water balance resulted in all 3 studies. At the end of 24 hours the addition of 0.3% NaCl to 5% glucose (C) resulted in continuing diuresis despite an increase in serum sodium concentration and an essentially unchanged serum osmolal concentration.

Urine osmolality and electrolyte concentration Except during the early period of the infusion and before the establishment of diuresis urine osmolality tended to vary inversely with volume. The number of milliosmoles of solute in urine excreted during the 10 hour infusion period showed remarkable constancy for all 3 test solutions. Both subjects demonstrated a 5 per cent fall when 0.15 per cent NaCl/glucose was compared to glucose alone during the infusion period (Figs 1 & 2).

The highest hourly rates of excretion of sodium chloride and potassium were observed during the first hour of infusion with all test solutions in subject A1. After demonstrating a similar high level during infusion of glucose solution, subject Ro achieved highest hourly excretion rates at 2 and 3 hours after initiation of infusion for 0.15 per cent NaCl/glucose and 0.30 per

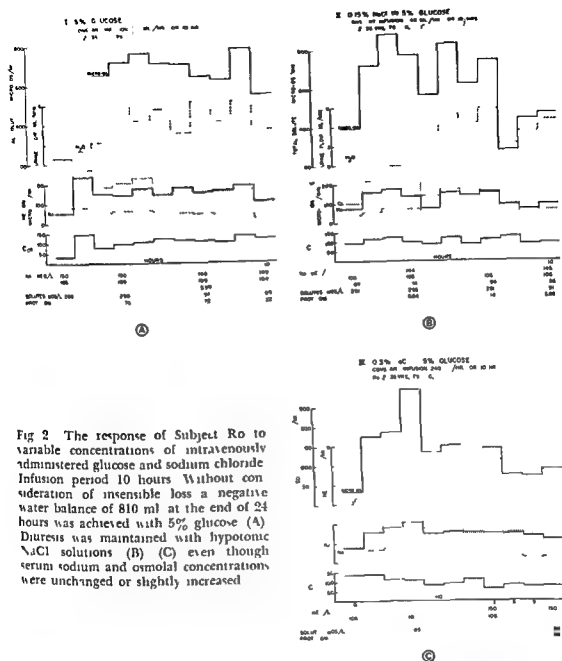


Fig 2 The response of Subject Ro to variable concentrations of intravenously administered glucose and sodium chloride. Infusion period 10 hours. Without consideration of insensible loss a negative water balance of 810 ml at the end of 24 hours was achieved with 5% glucose (A). Diuresis was maintained with hypotonic NaCl solutions (B) (C) even though serum sodium and osmolal concentrations were unchanged or slightly increased.

cent NaCl/glucose respectively. The excretion of sodium chloride and potassium demonstrated no striking changes during the infusion period when the 3 solutions were compared, although there was a slight decrease in all 3 ions when 0.15 per cent NaCl/glucose solution was given. There was a sharp increase in electrolyte excretion during the subsequent 14 hours after inclusion of NaCl in the infusate. Both subjects excreted almost all of the load of NaCl during a 24 hour period when 0.15 per cent NaCl/glucose was given, and subject Ro apparently had similarly excreted the salt load provided by 0.30 per cent NaCl. Subject Al retained a part of the salt infused by 0.30 per cent NaCl/glucose as his excretion rates with this solution tended to parallel those of the more dilute saline solution.

Serum electrolyte concentration, osmolality and protein changes. In gen-

erul the changes in serum sodium concentration paralleled those of osmolality but did not always occur in the same degree (Figs 1 & 2) Chloride and potassium changes were variable and appeared to be of little significance The decrease in serum osmolality and sodium levels was not so marked with 0.15 per cent NaCl/glucose as with glucose alone and during the administration of 0.30 per cent NaCl/glucose these changes were minimal Subject Ro apparently was able to diurese even though the osmolal concentration and sodium levels were above those observed at the beginning of the study Subject Al demonstrated a more conventional decrease in osmolal concentration and sodium When administered 0.30 per cent NaCl/glucose however, he was able to maintain diuresis despite an increase in serum sodium concentration no essential change in the serum osmotic pressure

Serum protein levels fell consistently regardless of the type of solution used At times these changes were marked being more than double the fall of serum sodium and osmolality This fall in serum protein occurred even when an increase in osmolality and sodium levels were found

DISCUSSION

The addition of 0.15 per cent NaCl and 0.30 per cent NaCl to 5 per cent glucose did not prevent the negative water balance observed when 5 per cent glucose alone was given although the degree of negativity was less when salt was added During the period of infusion the number of mOsm excreted in the urine remained remarkably constant for all 3 test solutions During the subsequent 14 hours however the sodium load imposed by 0.15 per cent NaCl was essentially excreted and the load provided by 0.30 per cent NaCl was excreted in one subject and partially retained in the other

It has been stated that no other mechanism excepting the depression of antidiuretic hormone activity responds to hypotonicity of body fluids⁹ If such is true then some other mechanism is responsible for the diuresis observed in certain of these studies that demonstrated little concomitant change in or actual increase of the serum osmolality and sodium concentration Certain reservations must be made however as hourly serum osmolal concentrations were not obtained

Welt and Nelson⁹ observed in their studies that the fall in serum proteins accompanying administration of intravenous 5 per cent glucose and water to normal subjects did not occur when similar amounts of tap water were given by mouth As an explanation they suggested the excretion of electrolytes into the gastro-intestinal tract in response to a large volume of water The fall in serum proteins in these studies has been too great to be explained by simple dilution and furthermore occurred during periods of relatively increased sodium concentration and increased serum osmolality This suggests that proteins may have escaped in some manner probably into the interstitial fluids of the body If there is a relationship between such relative hypoproteinemia and diuresis of intravenous fluids under the conditions of these studies its confirmation awaits further investigation

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THE TREATMENT OF EXPERIMENTAL CEREBRAL EDEMA BY INTRAVENOUS HYPERTONIC SOLUTIONS*

RAYMOND CLASEN SYLVIA PANDOLFI FELIX MARTIN
AND C BRUCE TAYLOR

The role of cerebral edema in the basic patho physiology of cerebral trauma and vascular accidents has remained by and large unclarified and the treatment of cerebral edema by intravenous hypertonic solutions is still controversial This paper is an attempt to seek a partial answer to these questions by means of acute closed cerebral lesions produced by freezing through the intact skull The technique was originally described for rabbits¹ The work in this paper was done on dogs The lesions produced by this means consist of focal circumscribed areas of hemorrhagic tissue destruction involving the cerebral cortex (Fig 1) and extending for a variable distance into the adjacent white matter (Fig 2) Histologically there was complete destruction of neuronal elements with perivascular hemorrhage in the area of gross damage A complete description of the gross and microscopic characteristics together with the method for production of lesions in dogs has been published previously² Definite foci of cerebral edema were noted in the white matter adjacent to the areas of gross destruction This could be demonstrated both by ordinary histologic methods and by staining with vital dyes

METHOD

Local closed cerebral lesions were produced in the posterior portion of the left hemisphere of adult dogs anesthetized with intravenous sodium pentobarbital The lesions were made by the application of isopentane frozen to the solid state by means of liquid air to the exposed intact skull The

*Rush Laboratories of Pathology and Surgical Research Presbyterian Hospital Chicago Illinois This work was supported in part by Contract AF16(600)628 monitored by The School of Aviation Medicine and in part by The Otto S A Sprague Memorial Institute



Fig 1 A small lesion in the right hemisphere and a moderately large lesion in the left hemisphere produced by freezing through the intact skull. The margins of the lesions are sharp allowing an accurate determination of the surface area

intracranial pressure was measured by means of a needle in the cisterna magna which was connected with a Statham strain gauge. The animals were sacrificed at times varying from 150 to 600 min after the production of the lesion. At the conclusion of each experiment the brain was removed and weighed. The surface area of the lesion was determined by means of transferring a template to graph paper and counting the squares. Each hemisphere was severed and reweighed separately. The water content was determined by drying to constant weight in vacuo after acetone extraction. The iron content of both the residue and extract was determined by the ortho

Fig 2 A cross section of the brain shown in Figure 1. The larger lesion extends for some distance into the white matter but the border is still well defined. Edematous changes were noted in the white matter adjacent to the area of destruction



phenanthroline method after dry washing with calcium carbonate.³ The blood volume equivalent of this iron was then calculated by comparison with the concentration of iron in the venous blood just prior to the time the animal was sacrificed.

Five groups of animals were studied by this means. The first was a control group of 7 animals in which the normal weight water content and blood volume (expressed as the blood equivalent of hemisphere iron of canine cerebral hemispheres) was determined. The second group consisted of 9 animals with lesions in the left cerebral hemisphere. The third group consisted of 6 animals with unilateral left cerebral lesions treated by the intravenous administration of either 50 per cent glucose or 25 per cent human albumin. The fourth group consisted of 7 animals with unilateral cerebral lesions from 200 to 300 min. old treated with either 12 per cent or 20 per cent hypertonic dextran.* The lesion size as determined by the surface area damaged per unit weight of the brain was statistically the same at the 5 per cent level of probability for all groups. The fifth group consisted of 5 hemispheres removed from 4 normal anesthetized dogs that had been given intravenous hypertonic dextran.

RESULTS

The chemical results are given in Table 1 in terms of the means and estimated standard deviations of the groups. Student's *t* test with 5 per cent as the level of significance was used to compare figures. The results from Group 1 indicate that normal canine hemispheres from the same animal do not differ significantly from each other with respect to water and blood content as determined by the methods used.

Table 1 Water and Blood Contents of Canine Cerebral Hemispheres

GROUP	WATER CONTENT (ml 100 gm TISSUE)		BLOOD EQUIVALENT OF HEMISPHERE IRON (ml 100 gm TISSUE)	
	RIGHT	LEFT	RIGHT	LEFT
1	78.53±0.42	78.37±0.39	4.72±0.35	4.65±0.23
2	78.61±0.29	79.55±0.33	4.55±0.38	8.10±0.66
3	78.20±0.54	79.76±0.51	6.71±0.23	9.45±0.94
4	80.25±0.49	81.52±0.50	6.52±0.62	8.62±1.14
5	78.91±0.70		6.85±0.82	

In Group 2 animals with untreated left hemisphere lesions a significant increase in blood and water content on the damaged side is seen. Comparison of the increments of blood and water in each animal indicates that they are independent variables ($r = 7.7 \times 10^{-4}$ showing no correlation). Furthermore there was a direct relationship between the water increment in the damaged hemisphere and the size of the lesion ($r = 0.81$ significantly greater than zero at the 5 per cent level of probability) while no correlation was seen when the blood increment was compared to the lesion size ($r = 0.09$). The lesions were associated with an increase in intracranial pressure which

*The dextran was supplied through Dr. Homer Staveland of The Commercial Solvent Corporation.

varied from 120 mm to 480 mm and was sustained for as long as 10 hours. While the pressure varied it never permanently returned to normal.

The increased intracranial pressure was reduced by the use of hypertonic glucose. Hypertonic albumin failed to reduce the intracranial pressure to the low levels obtained with glucose. The chemical results indicate an increase in cerebral blood volume but no effect on either the water content of the undamaged hemisphere or the water increment in the damaged hemisphere (Group 3).

Following the use of hypertonic dextran (Group 4) there was a slight increase rather than decrease in intracranial pressure. The chemical findings also indicate an increase in cerebral blood volume with no effect on the water increment in the damaged hemisphere. There was however a significant increase in the water content of the undamaged hemisphere. In order to determine whether or not this was due to the presence of the lesion dextran was given to normal animals. There was little change in intracranial pressure. The apparent difference in water content of the right hemisphere in Groups 4 and 5 is not statistically significant. The effect of dextran on the water content of damaged tissue is apparently no greater than its effect on the water content of undamaged tissue.

DISCUSSION

The increment of water on a unit weight basis in the damaged hemisphere is relatively small but it must be emphasized that this is no measure of volume increase since it depends entirely upon the difference in water content of edema fluid, blood, and the brain substance in which these changes are taking place. A better measure of the volume factor is the increase in total weight of the damaged hemisphere which averaged about 8 per cent while the average increase in water was only 1 per cent. This overall increase in volume occurring locally in a closed system might be expected to have a considerable effect on intracranial dynamics and cerebral function.

The effects of hypertonic fluids on the other hand might best be measured by considering the most labile component of cerebral edema, namely its water content. It has been shown in these experiments that hypertonic solutions have no effect on this factor. The effect of all hypertonic solutions in increasing the cerebral blood volume is in keeping with the generally accepted notions on increased intravascular osmotic pressure and fluid movements. Since the blood volume was increased and the water components of the brains were not affected the pressure reduction can only be explained on the basis of the Monroe-Kellie Doctrine⁴ by assuming that spinal fluid was withdrawn from some reservoir not in the cerebral hemispheres.

Dextran was not only ineffective in treating the localized cerebral edema in these experiments but its use led to a significant increase in the total water of the undamaged hemisphere. Whether or not such a change has any physiologic significance remains to be determined. On the basis of these findings we have come to the conclusion that cerebral edema of the type produced here is not diminished by intravenous hypertonic solutions. Since dextran is often used to treat hemorrhage or shock in patients with intracranial injury, our data also indicate that the potential danger of its use in such cases should be examined.

CONCLUSIONS

1 Unilateral cerebral lesions produced by freezing through the intact skull in dogs are associated with an increase in blood and water in the damaged hemisphere and a state of intracranial hypertension

2 Hypertonic glucose and albumin reduced, temporarily, the increased intracranial pressure even in the face of an increased intracerebral blood volume without affecting the increment of water in the damaged hemisphere and the water content of the undamaged hemisphere

3 Hypertonic dextran fails to reduce the increased intracranial pressure seen with these lesions and in addition causes a significant increase in the water content of the undamaged hemisphere without affecting the water increment of the damaged hemisphere The cerebral blood volume is increased to the same degree as seen with hypertonic glucose and albumin

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INTERPRETATION OF THE SERUM POTASSIUM CONCENTRATION IN PATIENTS WITH ACID-BASE IMBALANCE*

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It has long been suspected that acid-base imbalances induce transfers of potassium between the cells and the extracellular space. However, there are conflicting opinions as to the direction and magnitude of these transfers. Inasmuch as clinicians must of necessity use the serum potassium concentration as a guide to potassium therapy, it is of importance to clarify the direction and magnitude of change in serum potassium concentration induced by alteration of extracellular pH. The present study of 5 human subjects demonstrates that acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium.

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METHOD

Five patients were selected for study because of alterations of extracellular pH of either metabolic or respiratory origin. Extracellular pH was manipulated by infusions of NaHCO_3 , NH_4Cl , HCl and on one occasion the concomitant use of 8 per cent CO_2 inhalation. Effort was made during the period of pH change to minimize changes in the body potassium as related to nitrogen.

Whole blood pH was measured microbially on femoral venous blood within 10 minutes after sampling. pH measurements were made at 38°C using a Cambridge pH meter with glass electrode. Venous rather than arterial pH was determined because central venous pH was considered to more accurately reflect extracellular pH. Plasma potassium concentrations were measured on the same specimens using a Bard flame photometer with lithium internal standard. Potassium and nitrogen balances were measured by standard methods.

RESULTS

The effect of extracellular pH change on the serum potassium concentration is illustrated by the course of events in patient H. M. (Fig. 1). The patient had respiratory alkalosis associated with hepatic coma. Extracellular pH was manipulated by a combination of HCl infusion and carbon dioxide inhalation. No potassium was given during the 5 day study. The reciprocal relationship between extracellular pH and serum potassium concentration is apparent throughout the study.

Data from 9 experimental periods of study of 5 patients are presented in Table 1. In all instances acidosis increased and alkalosis decreased the serum potassium concentration. Changes in total body potassium in excess of nitrogen were small (Column 5 of Table 1). Furthermore, in 6 of the 9 periods of study the change in potassium balance was in the opposite direction to the change in the serum potassium concentration. Thus, changes in potassium balance were not the cause of the observed changes in serum potassium concentration.

In the last column in Table 1 change in serum potassium concentration has been calculated for each 0.1 unit pH change in the extracellular space.

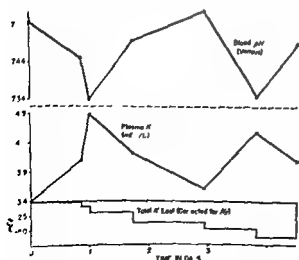


Fig. 1. Serial changes in blood pH, plasma potassium concentration and potassium balance in patient H.M.

Table 1 Relationship Between Changes in Intracellular pH and Change in Serum Potassium Concentration in 9 Experimental Periods in 5 Human Subjects

PATIENT	PERIOD	ΔK	ΔpH	ΔK_1	$\Delta K \text{ 0.1 } \Delta pH$
H M	1	+0.7	-0.10	-1	0.70
	2	+1.0	-0.10	-6	1.00
	3	-0.9	+0.16	-10	0.56
	4	-0.6	+0.08	-18	0.75
	5	+1.0	-0.23	-6	0.44
J R	1	+0.1	-0.13	-5	0.39
H I	1	-2.1	+0.18	+32	0.30
J N	1	-1.9	+0.15	0	1.27
J D	1	-2.1	+0.17	-15	0.51

ΔK_1 = Change in the serum potassium concentration in mEq/L

ΔK_b = Change in potassium balance in excess of nitrogen (K/N ratio = 3 mEq/gm)

The mean change for all periods was 0.68 mEq/L change in serum potassium concentration per 0.1 unit change in extracellular pH. The range was 0.39 mEq/L to 1.27 mEq/L change in the serum potassium concentration per 0.1 unit pH change.

DISCUSSION

The serum potassium concentration in the absence of acid base imbalance accurately reflects intracellular potassium stores¹. Alkalosis decreases and acidosis increases the serum potassium concentration in relation to the intracellular stores. The greater the deviation of extracellular pH from normal the more the serum potassium concentration fails to reflect the intracellular stores. This deviation may be so great that in severe alkalosis there may be a very low serum potassium concentration with essentially normal potassium stores. In severe acidosis a high serum potassium reflects normal potassium stores, a normal serum potassium reflects a moderate potassium depletion and a low serum potassium reflects a profound depletion. The muscle analyses of Mudge and Vislocky offer support for these concepts.²

If the serum potassium concentration is to be interpreted to reflect the level of intracellular potassium stores either there must be no acid base disorder present or correction must be made for deviations of up to 3 mEq/L in the serum concentration which can be induced by alteration of extracellular pH.*

Although the serum potassium concentration fails to reflect potassium stores in the presence of acid base imbalance it remains the best practical guide to therapy. Whether initial hypokalemia is secondary to intracellular potassium depletion, alkalosis or both potassium chloride is good therapy. If potassium depletion is the dominant cause of the hypokalemia potassium is needed. If alkalosis is the dominant cause of hypokalemia potassium chloride will help compensate for increased renal excretion of potassium which may accompany alkalosis³ also provision of chloride ion even though the

The effect of alterations of extracellular pH on the relationship between extracellular and intracellular potassium is probably related to intracellular buffering and therefore ultimately dependent upon intracellular pH change. However since the latter cannot be measured the relationship must be studied in terms of extracellular pH.

potassium may be excreted will aid in the repair of the alkalosis. Conversely whether initial *hyperkalemia* is secondary to acidosis intracellular potassium excess or both potassium restriction would be indicated because of the danger of producing cardiotoxicity. Restriction is also indicated because hyperkalemia would represent at most minimal potassium depletion even with severe acidosis. However it is important to again emphasize that the more severe the acidosis the less the serum potassium concentration reflects the intracellular stores. Thus in the presence of severe acidosis a normal initial serum potassium concentration represents significant depletion and warrants potassium therapy as soon as an adequate urine volume permits. Rapid correction of the acidosis such as occurs in the treatment of diabetes acidosis causes a rapid fall in the serum potassium concentration. This fall makes early therapy with potassium perfectly safe and gives additional warning of potassium depletion.

The results of this study have at least two additional implications. 1) Extracellular pH must be controlled in all experiments investigating other factors which may change the serum potassium concentration. 2) Avoidance or correction of acidosis will aid in control of hyperkalemia in patients with renal failure.

SUMMARY

1 The effect of acid base imbalance on the serum potassium concentration has been studied in 5 patients.

2 Acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium.

3 These observations permit more accurate estimation of intracellular potassium stores from the serum potassium concentration in patients with disturbance of acid base imbalance.

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METABOLIC STUDIES ON INFANTS WITH ENTEROSTOMIES*

WILLIAM R. RICHARDSON

The use of enterostomy in the management of small bowel obstruction requiring resection in infants has been frequently condemned but its revival as part of a Mikulicz type two stage procedure during the past 7 years at Boston Children's Hospital has been associated with a gratifying increase in survivors. Most surgeons have avoided its use even as a temporary measure because of disastrous losses of fluid and electrolytes especially in the newborn infant. We have found that large enterostomy losses can be adequately replaced and need not constitute a contraindication to the use of this technique. The purpose of this study was to define the patterns of enterostomy function and the extent of the gastrointestinal losses of fluid and electrolyte. Time does not permit discussion of other aspects of nutritional problems and care here.

METHOD

Nutritional data have been recorded on the 180 cases of small bowel obstruction managed by this method in the past 7 years. Most of the patients were under 1 year of age and over half were newborn. Diagnoses were congenital atresia 50, meconium ileus 10, intussusception 30, congenital stenosis 12, volvulus perforated Meckel's diverticulum duplication hernia neoplasm and miscellaneous totalling 48 others. Many serum chemistries extrarenal loss analyses and spot observations on gastrointestinal function were made and a detailed metabolic balance was performed on an infant with congenital ileocecal atresia. Representative charts have been studied extensively with calculations of losses based on theoretical considerations as well as measured intake and body weight.

The balance and analytic techniques employed were those of the Children's Hospital Metabolic Unit under the supervision of Dr. William M. Wallace of the Department of Pediatrics.† Collection and measurement procedures have been described elsewhere.¹ Ileostomy losses were measured by gravimetric technique for volume and water content and electrolyte losses were calculated by analyses of the equilibrated wash fluid. Recoveries by this method were accurate within 3 per cent for inorganic ions.

Balance analyses were performed as follows: Sodium and potassium in 0.2 ml. serum or 1.0 ml. urine by flame photometry;² chloride in stool drainage and urine by a modification of the method of Wilson and Brill³ and in serum by the method of Van Slyke and Hiller⁴; blood pH by the method of Sendroy and Hastings; and total carbon dioxide by the method of Van Slyke and Neill.⁵

RESULTS

Figures 1 and 2 present balance data on infant R.S. from a 12 day period beginning the day after ileostomy and ending 4 days after closure when normal stools were appearing.

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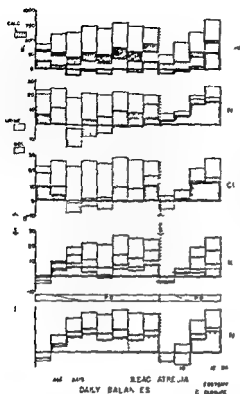


Fig. 1 Daily sodium chloride potassium nitrogen and fluid balances in patient R S. Urinary and ileostomy (stool) losses are charted downward from the total intake line. Positive balances are the dark areas above and negative balances everything below the zero line. Column width has no significance. Daily fluid balance minus calculated insensible weight loss ($1000 \text{ g/m}^2/\text{day}$)² equals theoretical body weight change (not corrected for water content).

R S had lost 13 per cent of his birth weight of 3.1 kg by the time of admission at age 9 days. At operation a short atretic distal ileac segment was resected. The ileostomy spur was crushed by the eighth day and extraperitoneal closure under cyclopropine anesthesia was performed on the ninth day. He was discharged home 13 days later weighing slightly more than birth weight. Oral intake was entirely Nutramigen* after the third day. Extracanal losses were almost entirely intestinal and insensible.

Nitrogen losses were small and balance was soon steadily positive. Only water and electrolytes need concern us in such an acute fluid disturbance.

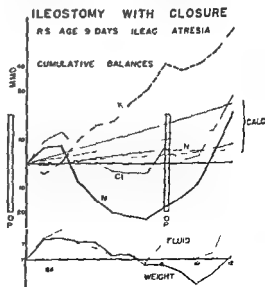


Fig. 2 R S cumulative balance and body weight curves compared with theoretical normal growth retention curve.

*Nutramigen—a casein hydrolyate mixture. Mead Johnson & Co.

Daily electrolyte losses via ileostomy were remarkably similar even with varying volumes. Measured extrarenal sodium and potassium losses averaged 45 per cent of total output after the first day contrasted with 26 per cent for water, 28 per cent for chloride and 25 per cent for nitrogen. The briefly positive sodium and chloride balances in the beginning and preclosure days reflect decreased urinary losses.

Figure 2 attempts to present a more composite picture by charting cumulative balance curves which have proved of special value in studies of rapidly growing infants. No conclusions can be drawn concerning the metabolic response to the first operation but certainly the ileostomy closure 11 days later constituted minimal biological stress.

R.S. had a more uneventful convalescence than the average. His low ileostomy demonstrated fairly effective water and electrolyte absorption but his renal losses, especially of water, were excessive perhaps due to immaturity.

Figure 2 also indicates the pattern and magnitude of the water exchanges. The calculated and observed body weight curves coincide remarkably except for a decrease in the measured urinary output on the day before closure.

The ileostomy drainage volume levelled off under 200 ml. on exclusively oral intake (Table 1). It contained more sodium than chloride but was hypotonic for both. The potassium content was consistently high with values 5 to 6 times the extracellular fluid concentration and about $1\frac{1}{4}$ times the intake concentration.

Table 1 Composition of Gastrointestinal Loss

SOURCE	DAILY VOLUME	CONCENTRATION IN mEq./L.		
		Na	K	Cl
R.S. Day #2—Ileostomy	70	152	81	54
R.S. Day #3—Ileostomy	133	94	50	42
R.S. Day #4—Ileostomy	151	73	36	32
R.S. Day #5—Ileostomy	132	93	56	39
R.S. Day #6—Ileostomy	194	56	50	37
R.S. Day #7—Ileostomy	155	51	46	34
R.S. Day #8—Ileostomy	140	67	48	49
R.S. Average Days 4 thru 8	160	66	47	35
Tube suction drainage (stomach) (infants under 6 months)	12 specimen patients	73 (50-100)	11 (3-21)	95 (50-114)
Tube suction drainage (jejunum) (infants under 6 months)	8 specimen patients	94 (50-100)	31 (2-45)	94 (50-105)
Oral intake—Nutramigen (average of several batches)		29	31	35
Evaporated milk (modified) formula		26	30	31

In a large number of cases of various types with varying patterns of intestinal function, theoretical volumes and sodium contents of enterostomy drainage have been calculated. Table 2 presents data from 9 representative cases. The first infant should represent some kind of a record water turnover or exchange rate. During his wettest consecutive 4 day period, his average

Table 2 Representative Cases—Intake and Output Data

INITIAL	CR	DO	LT	PF	JP	WB	AG	PA	EC
AGE DAYS	60 d	1	9	2	3	3	1	70	1
WEIGHT kg	33 kg	32	22	24	20	32	24	40	27
DIAGNOSIS	LOW ILEA STENOSIS	HIGH ILEA ATRESIA	MID ILEA ATRESIA	LOW ILEA ATRESIA	HIGH ILEA ATRESIA	MID ILEA ATRESIA	MECON ILEUS	LOW ILEA STENOSIS	MECON ILEUS
Length of Period *	1	1	11	3	3	4	3	4	4+
Length of Period B†	12	1	8	9	4	17	6	9	8+
INTAKE									
Rate ml/kg/d	306	342	312	260	183	182	177	170	151
Rate ml M ² /d	6000	7090	4560	3910	2860	2920	2460	2890	2400
% Intravital	43	26	43	31	0	10	9	1	0
Average Na conc	52	46	39	38	26	28	30	20	26
ENTEROSTOMY OUTPUT‡									
Rate ml/d	1000	590	385	310	184	157	118	211	70
Rate ml/kg d	310	180	176	130	74	50	49	53	20
Rate ml/M ² /d	5000	2700	5340	2000	1150	830	710	910	400
Average Na Conc.	51	65	57	53	30	58	80	47	83

*Period A is early postoperative before full G I function established

†Period B follows full ileostomy closure (functional plateau)

‡Output values are calculated assuming liberal daily average skin lung and kidney expenditure of H₂O=2000 ml/M² Na=50 mEq/M²

daily intake was 514 ml/kg or 8770 ml/M (13 per cent parenteral) with average sodium concentration 7 this translates to an enterostomy loss of 1370 ml/day or 400 \pm ml/kg/day. The adult equivalents calculated on any basis are startling.

Most of the survivors in the newborn group fitted a generally similar clinical pattern. An average case would show: 1 Nasogastric tube suction 1-2 days. 2 Full oral formula by the fourth or fifth day. 3 Full parenteral intake for 2 to 3 days tapering to an occasional supplement after full enterostomy function was established. 4 Small gradual loss of weight reversed after oral intake was resumed following closure with discharge weight about equal to birth weight. 5 Mild physiological disturbance by operative closure on the 7 to 12th day. 6 Enterostomy output volume plateau of 250-300 ml/day (equivalent to approximately 100 ml/kg or 1500 ml/M²/day). 7 Enterostomy drainage concentrations varying in mEq/L Na 60-70 Cl 45-50 K 35-40. 8 Average daily enterostomy losses (plateau) 250 gm of water 16 mM of sodium 12 mM of chloride and 9 mM of potassium. 9 Intestinal transit times (carmine) averaged about 1 hr with a range of 10 min to 2½ hr.

DISCUSSION

The volume and composition of these enterostomy losses are suggestive of those recorded for infants with moderately severe diarrhea. Although a few spot analyses of drainage from parenterally fed infants show a composition similar to that of orally fed infants and it is known that stools from fed and fasted infants with diarrhea are generally similar, comparable complete balances are needed to permit evaluation of the role of intake in the determination of the enterostomy loss. The wide range of losses points up the importance of frequent quantitative checks on clinical observation. These can usually be chiefly intake volumes and body weights but the occasionally severely ill or profusely draining infant will need methods for volumetric collection of urine, weighing of ileostomy dressings and even electrolyte analyses of loss samples.

The commonest metabolic imbalance encountered in the young infants was hypertonic dehydration due to a relative water deficit probably occurring through the combined effects of low intake and loss of hypotonic fluid from skin, lungs and intestinal tract.

SUMMARY

- 1 Enterostomy losses in infants need not be disastrous.
- 2 Although marked variations occur a general pattern of clinical gastrointestinal function has characterized the survivors.
- 3 Extrarenal loss analyses have confirmed the generally hypotonic nature of infant gastrointestinal secretions and have re-emphasized the value of frequently weighing the infant and his output.

Acknowledgement: I am grateful for the opportunity and encouragement provided by Dr Robert E. Gross on whose surgical service these patients were studied during my period of residency and research fellowship.

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THE EFFECT OF 19 NORTESTOSTERONE CYCLOPENTYLPROPIONATE ON NITROGEN BALANCE AND BODY WEIGHT IN POSTOPERATIVE PATIENTS*

WILLIAM E ABBOTT STANLEY LEVY HARVEY KRIFGER JEFFREY W BENSON AND C JACKSON RAYBURN

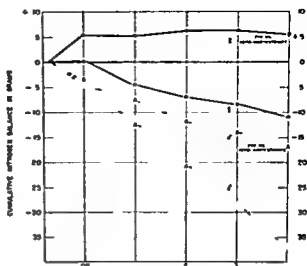
The large nitrogen deficits which have been reported to occur in surgical patients maintained on a starvation regimen (e.g. 1 to 3 L of 5 to 10% hexose solution/day) can be minimized or even negated by giving increased amounts of both nitrogen and calories.¹ In an individual of small stature (40 kg or less) it is possible to administer a sufficient caloric (35 to 10) and nitrogen (0.25 to 0.3 gm) intake on a per kg basis by using 2500 to 3000 ml of a 5 per cent protein hydrolysate solution containing 10 per cent hexose. However it is difficult to meet even the minimal caloric requirements (25 to 30 calories/kg/d) of the adult surgical patient who weighs more than 50 kg by use of only protein hydrolysates and sugar solutions. It has been shown previously^{1, 2, 3} that nitrogen wasting and weight loss can be minimized in the postoperative patient by administering a fat emulsion intravenously as a source of additional calories. But since intravenous fat emulsions are not readily available at this time the possibility was investigated of achieving reduced nitrogen deficits in surgical patients by giving them adequate amounts of nitrogen and modest caloric intakes (15 to 25 calories/kg of body weight) plus an anabolic hormone 19 Nortestosterone Cyclopentylpropionate†

Metabolic balance studies were carried out on 13 gastrectomized patients 7 males and 6 females who were maintained on adequate nitrogen and modest caloric intakes. Two of the males and 3 females also received an intra

*From the Department of Surgery, Western Reserve University School of Medicine and the University Hospitals of Cleveland, Cleveland, Ohio. This work was supported in part by grants from the National Institutes of Health, U.S. Public Health Service (A 760 (C3)) the Baxter Laboratories Inc., Morton Grove, Illinois, The Upjohn Company, Kalamazoo, Michigan, and the John A. Hartford Foundation.

†19 Nortestosterone Cyclopentylpropionate (Depo Nortestonate) was supplied by The Upjohn Company, Kalamazoo, Michigan.

Fig. 1 The average cumulative daily nitrogen balance for 7 male and 6 female subtotally gastrectomized patients who were maintained on comparable caloric and nitrogen intakes. Two of the males and 3 of the females received 250 mg of Depo nortestosterone



muscular injection of 250 mg of Depo-nortestosterone 1 or 2 days before operation. The details of the methods used in carrying out the metabolic balance studies have been reported elsewhere.¹⁻³

RESULTS

The average 5 day cumulative nitrogen balance including the day of operation and next 4 postoperative days for these 13 patients are shown in Figure 1. The 7 male patients were all given 0.15 to 0.2 gm of nitrogen and 20 to 25 calories/kg of body weight.

The 5 male patients who did not receive the Depo nortestosterone showed 5 day cumulative nitrogen deficits ranging between 33.7 and 39.4 gm or an average deficit of 36.7 gm. The postoperative temperatures of these 5 patients were essentially normal. The 2 patients who received 250 mg of Depo-nortestosterone preoperatively had only a 1.5 and 1.8 gm nitrogen deficit during this 5 day period in spite of pulmonary atelectasis and a fever of 38 to 39°C which persisted for 3 and 4 days. The average 5 day cumulative nitrogen deficit for these 2 patients was 16.7 gm. The 5 men who were maintained on modest caloric and nitrogen intakes lost between 3 to 5 pounds by the fifth postoperative day. The 2 men who had comparable operations and nutritional intakes and also received Depo nortestosterone (250 mg) showed no significant change in their weight postoperatively.

The average 5 day cumulative nitrogen deficit for the 3 female patients who had modest caloric (20 to 30 cal/kg) and nitrogen intakes (0.2 to 0.3 gm/kg) was 10.7 gm. In contrast there was an average 5 day cumulative positive nitrogen balance of 5.4 gm for the 3 females who received Depo nortestosterone. The 3 women who received the modest caloric and nitrogen intakes lost from 1½ to 3 pounds while those on a similar regimen plus Depo-nortestosterone gained 1 to 3 pounds over the 5 day postoperative period. The sodium and water balances in these patients revealed that the weight changes were not related to gains or losses of these substances. The sodium intake had been restricted to from 50 to 70 mEq in these patients and the 5 day cumulative sodium balances showed only slight deviations from equilibrium. In several other patients not included in this report who received Depo nortestosterone and 100 to 150 mEq of sodium daily positive sodium balances of from 20 to 60 mEq daily occurred for the first 6 to 9 postoperative days. These stud-

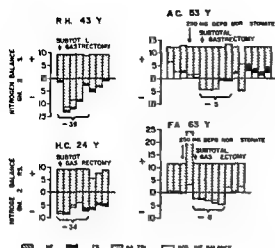


Fig 2 Nitrogen balance studies in 4 subtotally gastrectomized male patients. The degree of negative nitrogen balance is expressed by the extent of the column below the zero or base line. The cumulative 5 day postoperative nitrogen deficit is shown for each patient below the individual balance charts.

ies indicate that the partially gastrectomized patients who received modest caloric and nitrogen intakes showed smaller nitrogen deficits if they were given 250 mg of a long acting anabolic hormone (Depo nortestosterone) post operatively.

Figure 2 shows nitrogen balance charts for 4 of the male patients. 2 patients (F A and A C) received the Depo nortestosterone. The caloric and nitrogen intakes per kilogram of body weight per day were essentially the same for all 4 of these patients. It should be noted in the nitrogen balance chart of H C that this patient consumed orally significantly less nitrogen than he received intravenously for the first 5 study days. This diminished nitrogen intake was a result of his inability to eat more and this in turn prolonged the period of negative nitrogen balance. It has been noted that many patients are unable to take adequate oral diets during the first 5 to 10 postoperative days.²⁻³ On the other hand the patients who received the Depo-nortestosterone usually did not have postoperative anorexia.

DISCUSSION

The female patients had smaller nitrogen deficits than the male patients even though their nutritional regimens (per kg of body weight per day) were comparable. Similar findings were also noted in a previous study of subtotally gastrectomized patients who received no nitrogen and only a hexose solution for the first 5 postoperative days.¹ The male patients on such a starvation regimen had an average cumulative 5 day nitrogen deficit of 56.6 gm as compared to 30.0 gm for the female patients who likewise underwent a subtotal gastrectomy and received only 150 to 300 gm of hexose daily. Because of this apparent difference between males and females in their response to trauma one is not justified in comparing the nitrogen deficits of male postoperative patients with those of female postoperative patients.

No untoward effects were noted which could be attributed to the anabolic hormone. The female patients showed no androgenic effects. No accumulation of water and salt was noted if the sodium intake did not exceed 60 mEq/d.

SUMMARY AND CONCLUSIONS

From the data presented it is apparent that it is possible to reduce or negate the nitrogen deficit in the postgastrectomized patient by giving moderate

amounts of nitrogen and calories and employing an anabolic hormone such as Depo nortestosterone.

Male patients show a larger nitrogen deficit following surgery than do female patients undergoing similar operations when both groups are maintained on comparable intravenous regimens expressed on a per kilogram of body weight basis.

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EFFECTS OF THERMAL INJURY ON METABOLIC RATE AND INSENSIBLE WATER LOSS IN THE RAT*

ZELIG H. LIEBERMAN AND JAMES M. LANSCHÉ

This study was undertaken to investigate the influence of thermal injury upon the interrelationship of the metabolic rate and insensible loss of water in the burned rat. The rate of insensible loss of weight of rats bearing full thickness burns of 5 to 20 per cent of body surface significantly exceeds normal.¹

METHOD

Eighteen adult male Sprague Dawley strain rats were studied. Triweekly 1 hr measurements of oxygen consumption, carbon dioxide production, and insensible water loss were performed upon rats fasted for 12 hr using a Benedict indirect calorimeter.² The heat production expressed as calories per square meter of body surface for 24 hr was calculated from the measured oxygen consumption. Heat lost through the vaporization of water was determined by converting the measured extrarenal excretion of water to heat equivalents by employing the conversion factor of 586 calories/gm water. Following a 2 week control period full thickness dorsal burns of 5 to 20 per cent of body surface were produced with radiant energy from a parabolic reflector while the animals were anesthetized with ether. The rates of healing of the wounds were determined by the Carrel method.³ Rectal, cutaneous and wound temperatures were recorded with thermistors.

*From the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri. Based upon studies conducted under Army Contract No. DA 49-007-MD-636, Office of the Surgeon General.

The hair upon the dorsal surfaces of the body was clipped closely before the control period of observation.

The metabolic disturbances associated with a simple open wound were studied after the excision of skin from 10 to 30 per cent of the dorsal surface of the bodies of 3 anesthetized rats.

RESULTS

The metabolic rate (O_2 consumption ml /sq m /d) and insensible water loss (gm /sq m /d) were essentially constant for individual rats throughout the control period. Heat lost through vaporization of water represented 21 per cent \pm 2 of the total heat produced (See Table 1). This agrees very closely with Benedict's observations.⁴

Oxygen consumption and the rate of insensible water loss had fallen significantly by the third hour after injury. This decrease in oxygen consumption and insensible loss of water were directly related to the magnitude of the burn. (The larger the injury the greater the decline in metabolism and water loss.)

Between the second and seventh post burn days the insensible loss of water and oxygen consumption rose above pre burn levels, respectively attaining rates of 40 to 60 and 10 to 15 per cent above pre burn levels with burns covering 15 per cent of the rat. Concomitantly the proportion of heat lost through the vaporization of water increased from the control value of 21 per cent to 30 to 40 per cent of the total heat produced (Table 1). Associated with the separation of the eschar and the conversion of the injury to an open wound the insensible loss of water and metabolic rate increased further and attained respective levels of 100 to 150 per cent and 15 to 25 per cent above the pre burn levels (Table 1). While the open wound was large the heat lost by vaporization amounted to 10 to 60 per cent of all the heat produced. As the wound healed the rates of insensible loss of water and metabolism returned to the control levels.

There was no significant difference in rectal temperature during the pre burn and post burn periods except for a transient decrease in temperature noted immediately after thermal injury in the rats with the most severe burns. The surface temperature of the eschar was $0.8^\circ\text{C} \pm 0.2$ and of the open wound $1.0^\circ\text{C} \pm 0.2$ less than the surrounding skin.

One rat with a 15 per cent thermal injury was studied to determine the effect of placing an impermeable dressing (Sarin wrap) over the burn eschar. Coverage of the eschar with Sarin wrap was attended with an immediate decrease of the insensible water loss and metabolic rate to normal pre burn levels (Table 2).

Influence of Skin Excision. Open wounds of 10 per cent body surface were produced in 2 rats. There was an immediate three fold elevation of rate of insensible water loss and a one fourth fold increase in metabolic rate and the heat loss by vaporization of water amounted to about 70 per cent of total heat produced. Coverage of these open wounds with the skin removed previously was associated with an immediate return of the rates of insensible water loss and oxygen consumption to normal levels (Table 3 Rats A and B).

Excision of 30 per cent of the skin was followed by death of the rat within 18 hr. In this experiment the insensible water loss rose immediately and

Table 1 15°C Burns Comparison of Total Heat Production and Heat Loss Through Water Evaporation in the Burned Rat

ANIMAL	CONTROL				IMMEDIATE LOSS BURN				FACED				CHIN WOUND				HEATED			
	TOTAL		HEAT		HEAT		HEAT		HEAT		HEAT		HEAT		HEAT		HEAT		HEAT	
	VAL	(A)	H ₂ O	(B)	VAL	(C)	VAL	(D)	VAL	(E)	VAL	(F)	VAL	(G)	VAL	(H)	VAL	(I)	VAL	(J)
1	912	255	211	820	205	250	1022	508	385	1159	508	159	210	243	989	266	257	269	269	269
2	914	250	237	838	240	275	1029	380	370	1077	379	101	263	266	989	266	257	269	269	269
3	809	225	222	720	185	237	1091	125	989	1101	548	123	216	237	989	266	257	269	269	269
4	905	237	252	817	192	229	1036	124	102	1228	510	138	231	269	989	266	257	269	269	269

A Total heat production (Cal/m²/d) derived from oxygen consumption (18 x ml O₂=calories)

B Heat of vaporization of water (Cal/m²/d) derived from insensible water loss (0.6 x C H₂O=calories)

C Heat of vaporization of water per cent of total

Table 2 Influence of Saran Wrap Dressing on Insensible Water Loss and Metabolic Rate of the Burned Rat

DAY POST BURN	BURN COVERING	INSENSIBLE WATER LOSS GMS /M ² /24Hr	CALORIES PER SQ. METER/24 HR		WATER VAPORIZATION % OF TOTAL CALORIES
			TOTAL	VAPORIZATION WATER	
RAT D 15% BURN (PRE BURN)					
CONTROL	none	380	989	228	22.3
13	none	746	1251	454	36.2
13	Saran Wrap	430	1083	258	23.9

Table 3 Influence of Skin Excision of Metabolic Rate with Insensible Water Loss in the Rat

RAT	% SKIN EXCISION	RECTAL TEMPERATURE	C	INSENSIBLE WATER LOSS Gm /M ² /24Hr	CALORIES/SQ M/24 HR		WATER VAPORIZATION % OF TOTAL CALORIES
					TOTAL	VAPORIZATION WATER	
A Control							
4 hrs post excision	10%	36.4		441	912	264	28.9
Coverage with skin		35.8		1462	1297	892	68.7
		36.2		465	940	279	29.7
B Control							
3 hrs post excision	10%	36.1		414	981	248	25.3
Coverage with skin		34.3		1354	1152	812	70.2
		36.0		409	954	245	25.7
C Control							
3 hrs	30%	36.3		410	954	246	25.7
17 hrs		36.5		1251	1132	725	66.0
24 hrs		34.0		1915	1386	1152	83.1
48 hrs		32.1		1699	1180	1022	86.6
		32.0		1629	1111	974	87.6

quickly attained a rate five times normal and there remained until the animal died. There was a concomitant rise of metabolic rate of 10 per cent above normal; however, the percentage of heat loss by vaporization of water increased progressively and just before death it represented 87 per cent of the total heat produced by the animal (Table 3, Rat C). The rectal temperature decreased gradually during the 18 hr period while the animal shivered and drank water copiously. The water taken was equal to the water lost insensibly and the urinary flow was maintained.

DISCUSSION

Following a full thickness burn covering 5 to 20 per cent of the rat's body the rate of losing water through extrarenal channels is first decreased and then increased. The period of decreased insensible loss of water lasts 1 to 2 days corresponding roughly to the shock period. However, the animals were not anuric, maintained normal postural attitudes and moved about their cages during this time; consequently traumatic shock cannot be ascribed as the cause of the early depression of water loss.

Neither is this early depression attributable to changes in body temperature; the decline in cutaneous and rectal temperatures during this period were insignificant.

A decline in metabolism of endocrinal origin is a possible cause of the primary evanescent depression of insensible water loss. The turn over rate of I^{131} within the thyroid gland of burned rats is depressed during this same time (Reichlin and Lieberman unpublished data).

The period of elevated rate of loss of water extrarenally consists of two parts: the first lasting from the third to the fifth post burn day until full separation of the eschar (the eschar period) and the second beginning with the separation of the eschar and ending with complete healing of the wound (the open wound period). During the first part the rate of loss of water usually increases rapidly to a level where it remains fairly stable until the eschar separates; then the rate suddenly increases and subsequently gradually falls as the wound closes. During the period of elevated insensible water loss the metabolic rate is increased significantly. However, the rate of increase of oxygen consumption is generally calorically less than the increase of caloric loss incident to the increased rate of insensibly lost water. This suggests that the increased metabolic rate during the escharotic and open wound periods is secondary or stimulated by the increased rate of heat loss attributable to the evaporation of water through and from the eschar and the open wound.

In support of this view is the remarkably rapid drop of the metabolic rate (oxygen consumption) and evaporative loss of water to normal coincident with coverage of the open wound or eschar with a water impermeable dressing (Saran wrap). In addition, the same order of decline in loss of water insensibly and metabolic rate follows the reapplication of the excised skin to a surgically denuded area. Another observation adding support to this view is by lowering the environmental temperature to within the range of $2^{\circ}\text{C} \pm$ of the wound temperature; the closer is the coincidence of the increments of metabolism and heat loss through the vaporization of water.

These observations suggest that control of the environmental temperature and the construction of a dressing that will restrict but not prevent the transit of water through a wound would reduce significantly the metabolic de-

hands of a burned mammal and thereby permit an easier solution of the problem of post burn starvation.

The fact that a thermal injury remarkably changes the relationship existing between total heat loss and metabolic rate and the rate of loss of heat through the vaporization of water [heat loss by vaporization = 21 ± 2 per cent of total heat loss (normal)—heat loss by vaporization = 40 to 60 per cent of total heat loss (after larger burns)] obviates the use of the rate of insensible loss of water as a measure of the metabolic rate of burned mammals.

The faculty of a partially flayed mammal to maintain life is in part dependent upon the animal's ability to increase catabolism (heat production) so as to meet the immutable increase in heat loss attendant upon the vaporization of water from the wound surface. The increased heat loss associated with a 10 per cent flaying can be met, that associated with a 30 per cent skinning usually cannot be with environmental temperatures less than 28°C .

Thermally killed skin although hard and dry is much more permeable to water than living skin. The rise in rate of water loss attending burning takes place through it as attested by the immediate fall of insensible water loss to normal upon coverage of the eschar with Sui wrap.

The increased rate of vaporizational heat loss through burned skin and later from the open wound may be a most important factor in the genesis of the increased metabolism and the mortality of burned mammals.

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A PLANNED EVALUATION OF EARLY LACISION OF MORE THAN TWENTY FIVE PER CENT OF THE BODY SURFACE IN BURNS*

BRUCE G. MACMILLAN AND CURTIS P. ARTZ

Although survival times have increased in extensively burned patients, modern advances in therapy have not appreciably decreased the mortality rate in this group of patients. Once the initial period of resuscitation has been successfully managed, patients with large areas of full thickness burns are faced with the deleterious effects of invasive infection until complete skin coverage has been accomplished. The magnitude of this threat is clearly defined when mortality figures in burned cases are analyzed. Eighty-two deaths have occurred in the 1,000 burned patients treated at the Surgical Research

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Unit Brooke Army Medical Center Fort Sam Houston Texas between the years 1950 and 1956. Of these 82 deaths 16 (or 56 per cent) died from uncontrolled invasive infection. In the group of patients who died from septicemia the mean total body burn was 57 per cent the mean area of full thickness burn was 37 per cent and the mean burn index (third degree plus one half second degree) was 50.

In view of the present refinements in supportive therapy and the continuing threat of infection a careful evaluation of early removal of extensive areas of full thickness burns was planned.

METHOD

For this evaluation patients admitted since September 1, 1955 with an expected mortality of 50 per cent were selected. Individuals between the ages of 5 years and 55 years were studied. Only patients with 25 per cent or more of their body involved in full thickness burns and with a burn index in the range of 40 to 60 were included. Those patients admitted after the fifth post burn day were treated by conventional methods and those admitted prior to this time were excised. Excision of the areas of full thickness burn down to fascia was performed in one or two stages between the second and the fifth post burn days. Complete skin coverage was achieved either immediately or 2 days later by autografts and/or homografts. For immediate post burn resuscitation whole blood (0.5 cc./kg./per cent burn) was used for colloid replacement and lactated Ringer's solution (1.5 cc./kg./per cent burn) was used for electrolyte replacement. All patients were placed on penicillin and streptomycin during the first 5 post burn days. Antibiotic therapy for septicemia was determined from bacterial sensitivity and administered in the following dosage every 24 hours: cathomycin 1.0 gm, bacitracin 300,000 units, erythromycin 2.0 gm, chloromycetin 3.0 gm, and polymyxin 200 mg. All burned surfaces not excised were treated by the exposure method until healing or bacterial autolysis was noted. Areas of full thickness burn in the conventional group were prepared for grafting by aggressive debridement and saline wet dressings.

Technical Considerations. An early accurate appraisal of full thickness burn involvement can best be determined from the resultant loss of sensation. The inability to perceive pain usually signifies complete destruction of the skin; however, it must be borne in mind that some areas of the body are relatively insensitive such as the buttocks and the upper thighs. In excising areas of full thickness burn it is better to err on the side of over excision since the remaining third degree burn introduces infection, jeopardizes the graft take, and delays wound closure.

The depth to which areas of full thickness burn are excised likewise greatly influences primary wound healing. Incomplete removal of underlying fat results in a high percentage of graft failure. Since early complete wound closure is essential for the establishment of a protective barrier against bacterial invasion, all areas have been excised down to the underlying superficial fascia. With good hemostasis, grafting can be successfully accomplished on this base; however, if bleeding cannot be meticulously controlled, the incidence of graft failure rises precipitously. Under these circumstances, the wound is dressed over dry fine mesh gauze and grafting delayed for a period of 48 hours.

Bleeding is minimal if the excision is performed between the first and the third post burn day. After this period of time, bleeding is more profuse and its control more difficult. To minimize the operative time, all excisional procedures have been performed by utilizing 2 complete surgical teams. The average total operative time has been 2 hours. Cyclopropane anesthesia has been found to be the anesthetic agent of choice.

For skin coverage after the excisional period, all of the patient's uninvolved skin has been utilized at the first grafting procedure. Priority areas for coverage with autogenous skin are exposed fat tendons and joints. Excised areas which could not be initially covered with autogenous skin have been covered with homografts. Fresh cadaver homografts have resulted in the highest percentage of homograft takes. Viability of homografts has ranged from 21 to 57 days. When homografts have been rejected, autogenous skin obtained from healed donor sites or from healed areas of second degree burn has been used to complete the skin coverage.

RESULTS

Of the 80 patients admitted between September 1, 1955 and September 1, 1956, 13 patients were included in this study. Five cases were treated by early excision and 8 cases were treated by conventional methods. The results obtained in the excisional group are shown in Table 1. In this group of patients the area of full thickness burn ranged from 30 per cent to 13 per cent and the area of total body burn varied from 12 per cent to 53 per cent. The largest full thickness burned area excised at 1 operation was 32 per cent of the body surface. The interval between burn and initial excision varied from 1 to 2 days, excluding 1 patient who was excised on the fifth post burn day. Four patients underwent 2 procedures and 1 patient 1 procedure to remove all full thickness burn involvement. Blood lost during the excisional procedures ranged from 500 cc. to 2500 cc. Three cases were grafted at the time of excision and 2 cases were grafted 48 hours after excision. The lowest per cent take of autogenous skin grafts was 95 per cent and of homogenous skin grafts was 80 per cent. Complete autogenous skin coverage was accomplished in 1 of the 5 patients between the 28th and the 37th post burn days. None of these 4 patients developed septicemia. One patient (L.H., Table 1) died from a refractory pseudomonas septicemia on his 33rd post burn day before skin coverage could be accomplished. In this case there was insufficient uninvolved skin available for coverage with autografts when the initial homografts were rejected. Two of the 5 cases had uneventful recoveries. Two of the 5 cases expired as the result of unrelated secondary complications. One (H.I., Table 1) expired on his 30th post burn day after being given inadvertently an overdosage of morphine intravenously. The other (R.D., Table 1) died suddenly on his 42nd post burn day from a probable pulmonary infection.

In the group of patients treated by conventional means the area of full thickness burns varied from 40 per cent to 48 per cent and the total burned area varied from 40 per cent to 62 per cent. Two of the patients in this group survived and the remaining 6 patients died. Septicemia was the cause of death in all of the fatalities. In those patients who were grafted the initial skin was applied on the 18th and the 30th post burn day respectively. In the group of 6 patients dying from septicemia the initial positive blood

Table 1 Summary of Experience of Early Removal of Extensive Areas of Full Thickness Burns in Live Cases

PATIENT SEX AGE	BURN		SITE OF BURN	EXTENT OF EXCISION PER CENT	TIME DAYS BURN TO EXCISION	BLOOD		TIME DAYS EXCISION TO GRAFTING	GRAFT TAKE PER CENT	TIME DAYS		COMPLICATIONS	TIME DAYS BURN TO DEATH
	TOTAL	PER CENT				REFILL DURING EXCISION	COVER			RECOVER			
FH J	4	30	37½	Back Buttocks Anterior Thighs	30	1	1500 cc	2	Auto-9, Homo-9,	37	None		
AM M	4	36	40½	Ant chest Abdomen Rt arm	32	1	1500 cc	2	Auto-9, Homo-9,	40	Decubiti both heels		
FH M	5	43	49	Thighs	4	13	None	3	Auto-100	30			33
FH M	5	43	49	Buttocks Legs	40	2	1000 cc	0	Homo-80			Septicemia	
				Hands	3	4	1000 cc	0	Auto-8,	11			
FH M	12	30	37½	Arms Chest	15	2	750 cc	0	Auto-9, Homo-80	29		Morphine intoxication	30
				Abdomen Back	15	4	500 cc	0	Auto-9, Homo-80	28			
RD M	13	31	37	Legs	13	5	1500 cc	0	Homo-100	35		Irreparable pulmonary infarct	42
				Chest Rt Arm	18	9	2500 ml	0	Auto-9,	30			

Table 2 Summary of Experience in Eight Cases with Extensive Areas of Full Thickness Burns Treated by Conventional Methods

PATIENT SEX AGE	BURN EXTENT SURF. IN TOTAL %		BURN INDX	SITE OF BURN	TIME DAYS BURN TO CRUISING	TIME DAYS BURN TO CONTRACT	TIME DAYS BURN TO SURFECTIN	ORGANISM AND ANTIBIOTIC*	TIME DAYS BURN TO DEATH
M 1	10	30	3	Legs	32	90			
M 40				Buttocks					
				Back					
V 1	10	15	11	Back	24	135			
V 7				Abdomen					
				Arms legs					
M 1	55	10	15	Face arm	90			M pyogenes C B	93
M 21				Legs chest					
				Buttocks					
S 5	50	46	11	Arms legs				Pseudomonas I N	19
V 6				Abdomen					
								Enterococcus (resistant) M pyogenes C B	
R 11	57	90	11	Arms legs				M pyogenes B	24
M 32				Abdomen				Pseudomonas C I	
				Back					
					18			M pyogenes B	24
R 18	52	31	42	Arms legs				Pseudomonas A C	73
M 18				Abdomen					
R 10	00	29	12	Arms back				Unknown	11
M 9				Legs					
R 5	62	18	62	Arms legs		63		Pseudomonas A C	73
M 19				Back abdomen				M pyogenes B I Ca	

C - Chloromycetin A - Aureomycin B - Bacitracin I - Terramycin I - Irythromycin C - Cathomycin I N - I polymixin

cultures were obtained between the seventh and the 17th post burn days. Although all patients with septicemia were treated with the appropriate specific antibiotic only 1 patient (I S Table 2) responded initially to specific antibiotic therapy. On this patient's 51st post burn day, he developed a septic ileo-femoral thrombophlebitis which resulted in death on his 73rd post burn day. The organism most frequently recovered from the blood stream was *Micrococcus pyogenes* coagulase positive. In the group of patients developing septicemia death occurred from the 11th to the 33rd post burn day excluding the patient who developed septicemia late in his post burn course as the result of a septic ileo femoral thrombophlebitis. Skin coverage in the 2 survivors was accomplished on the 90th and the 135th post burn days respectively.

SUMMARY AND CONCLUSIONS

While the 5 patients treated to date by this aggressive attack on large areas of full thickness burns represent a small series certain observations can be made. No deaths in this series can be attributed to operative intervention in the early post burn period. Early excision and grafting of a full thickness burn wound involving 25 per cent or more of the total body surface can preclude invasive infection. Antibiotic therapy alone cannot protect the extensively burned patient against the rigors of bacterial invasion. The longer the interval between burn and excision the greater are the technical difficulties and the less effective the procedure. Experience gained to date with early removal of extensive areas of full thickness burns justifies its further evaluation.

PROTECTION AGAINST CONTACT AND FLASH BURNS*

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AND EDWIN W. SALZMAN

Each year many hundreds of man hours of productive effort are lost through one type of burn injury or another. For example contact burns from the accidental ignition of clothing occur with alarming frequency. A type of flash burn occurs in aircraft accidents or industrial explosions when liquid fuel explodes and burns. At yet a third level atomic warfare has brought with it another type of flash burn that is of importance to military and civil defense personnel.

Considerable effort has been exerted to study the characteristics of simulated atomic flash burns in order to understand the basic pathology and develop protective clothing for the armed forces or civil defense groups. However comparatively little experimental effort has been directed toward

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such common problems as contact burns or the type of flash burn encountered in the explosion of liquid fuel

Colebrook¹ has studied this problem clinically on the burn service of a large accident hospital. His interest was based on the clinical impression that the severity of injury following contact or flash burns varies widely and is to a large degree dependent on the clothing worn. A breakdown of the fabrics worn by 92 patients admitted to his service with severe thermal injury is as follows:

Cotton	61
Viscose Rayon	19
Wool	4
Cotton and Wool	3
Acetate Rayon	2
Cotton and Viscose Rayon	2
Viscose and Acetate Rayon	1

In his series all cellulose fibers were indicted with cottons by far the commonest offenders. Of the 4 patients wearing woolen materials these people were without exception either epileptics or aged persons who had fallen directly into an open fire. The absence of burns due to the newer synthetic fabrics can be attributed in part to their failure to support combustion but is also due to their comparatively infrequent use in 1951 at the time of the report.

The present study represents an attempt to quantitate and establish an experimental approach to evaluating the burn protection offered by various fabrics. Contact burns and flash burns simulating fuel explosions were selected for the study.

METHOD

Sprague Dawley rats weighing 250 to 350 gm. were clipped with surgical shears to remove the hair from thorax and abdomen. Twenty-four hours later the rats were weighed and anesthetized with pentobarbital sodium 30 mg/kg. The animals' trunks were covered with bands of fabrics stapled together to insure a snug fit.

In 1 series of 198 rats a standard wick of fabrics was prepared and heated to the ignition or melting point. If the fabric supported combustion it burned down to the animal's body and produced a contact burn. If it melted or failed to support combustion no burn resulted. Each of 32 fabrics was tested on 6 separate animals in this way.

The second series of 216 animals was prepared as above but each was held against an asbestos panel with a circular opening 2.5 cm. in diameter. The heat source—a Meker burner using natural gas—was applied to this opening and a temperature of 1204°C. (2200°F) was delivered to each animal for 3 seconds. Each of 33 fabrics was tested on 6 separate animals and 18 control animals wearing no fabrics were similarly exposed. The results in each series were recorded in the same manner. The burns were measured and the gross extent of each lesion was estimated at frequent regular intervals. The clinical course of each burn was recorded and in addition representative animals from each group were photographed in color at 24 hours, 7, 15 and 22 days after burning. Each animal was weighed weekly.

during convalescence. One typical member of each group of 6 rats was sacrificed at 24 hours and another at 15 to 18 days after exposure for microscopic study of the affected rat. The remainder of the animals were observed until completely healed and average healing times were determined for the various fabrics.

RESULTS AND DISCUSSION

In general 3 parameters of measurement were available: (1) average healing time, (2) gross pathological grading, and (3) microscopic pathological grading. The gross results were tabulated as +, ++, +++ on the basis of extent of lesions, hemorrhagic areas, margins, apparent depth, and rate of sloughing. This grading system proved to be especially useful in the flash burn series where each animal was exposed to a constant stimulus.

Microscopic grades of severity were recorded as 1 to 4 as follows:

(1) Superficial damage only, characterized by local autolytic change, thickening of the stratified squamous epithelium, and occasional small increases in the interstitial spaces.

(2) Coagulative necrosis of surface tissue extending to less than half the thickness of the corium. Some cases in this group showed destruction of the stratified squamous epithelium and all showed coagulative necrosis of the superficial collagen bundles.

(3) Coagulative necrosis extending the full thickness of the corium but not involving cutaneous muscle or subcutaneous tissue.

(4) Severe coagulative necrosis involving full thickness of corium as well as subcutaneous fat and muscle.

This type of classification is admittedly crude and depends on selecting a truly representative sample of burned tissue for examination. The use of several sections from each block tends to minimize this possible error but cannot completely remove it. The major changes seen in the sections taken at 15 days in comparison with those cut 24 hours after exposure are that the former show thickening of the epidermis and greater leucocytic infiltration. This likely represents organization and secondary bacterial invasion of greater or lesser degree.

Microscopically it was not possible to detect any significant morphological difference between burns of flash and non flash origin under these experimental conditions.

There was no histological picture typical of any specific fabrics tested. That is, there was no microscopic evidence that a burn involving nylon, for example, differed in any specific way from any other fabric. On early gross examination certain synthetic fabrics apparently melted into the skin when exposed to the Meker burner, but this did not appear to increase the severity of the burn or lengthen healing time.

For burns of equal depth, that is, equal histological grade, non flash burns healed faster than flash burns, this undoubtedly reflecting the fact that the latter involved much greater total area. This is also seen in an analysis of 20 grade 3 flash burns. The mean healing times for the 3 gross classifications (+ to +++) then present a straight line relationship showing that within a given histological grade (grade 3) the less extensive gross burns (+ to ++) heal more rapidly than do the more severe burns (++ and +++ to ++++). This supports the use of 3 parameters of measurement for extent

depth or healing time alone are not adequate to permit estimation of the other measures. Given any 2 parameters the third could be predicted with fair accuracy within general limits.

Utilizing these 3 parameters then in order of decreasing importance protection against this type of flash burn was found to lie in (1) weight or thickness of the material with heavy fabrics offering greatest protection (2) ability to conduct heat or chlr (a quality inherent in the material) (3) color with dark colors protecting less than light (4) flame retardent treatments.

Under experimental contact burn conditions wool, nylon, dacron and flame retardent treated cotton failed to ignite and no burn damage resulted. Cotton synthetic combinations burned sluggishly, but flame retardent treatment prevented this damage. Every sample of untreated cotton produced significant clinical burns.

Loose fitting garments allowed free circulation of oxygen between fabric and skin. The resulting burns were many times as severe as those seen with snug fitting samples of the same fabric.

CONCLUSIONS

This experimental study has emphasized the major qualities of fabrics which contribute to their ability to protect against thermal injury. It has also pointed out the failure of certain materials to protect and offers experimental evidence which supports the major clinical survey of a similar kind.

Colebrook¹ stated that in 1905 *The Lancet* stated that the days of flannelette should be speedily numbered because of its high flammability and the great number of accidents associated with its use. Fifty-one years later flannelette remains in wide use and flame proofing does not appear to be economically feasible or at any rate is not generally accepted. Furthermore we see no really active efforts to develop better substitutes or adequate flame retardent treatments. It is hoped that clothing manufacturers will come to appreciate the importance of this step and furthermore exercise judicious choice of the newer synthetic fabrics and synthetic cellulose combinations.

SUMMARY

Criteria for histological grading of thermal injury have been presented. In studies of flash burns and contact burns on rats grading based on these criteria have shown a positive correlation with gross pathological grading and with healing time. These parameters have been employed in the estimation of the protective value of various fabrics.

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EFFECT OF HORMONES ON THE METABOLISM OF SURGICAL PATIENTS*

HELENA CHIDLER EDWARD A. IRLE DONALD I. WEEKS JR.
AND JOHN M. BEAT

The catabolic response to surgery has been shown to be reversible to a degree which depends upon the extent of trauma and the continuous administration of sufficient nitrogen calories and potassium to cover the needs during the postoperative period.¹ It became apparent that a part of the catabolism or of negative nitrogen balance which occurred postoperatively was due to the withdrawal of nitrogen from the intake during this period. It was suggested that in the constant dynamic events occurring in the break down and repair of tissue a situation of stress does not necessarily delay or repress anabolism but may merely accelerate catabolism.

Testosterone has long been known to be anabolic and has been utilized in attempts to improve the nutritional state of certain patients.² The associated androgenic effect limited its usefulness. The development of a derivative of testosterone (17 α -ethyl 17 hydroxy 19 nor-4 androsten 3 one) known as Nilevar[®] with anabolic properties and without significant androgenic effect initiated the present studies. The purpose of the work was to determine whether postoperative catabolism could be further reduced by administration of this hormone.

It has been shown in this laboratory that testosterone does not affect the nitrogen balance postoperatively if nutrients are not supplied at the same time. In the present report 10 patients on a constant intake of nutrients throughout the operative period have been studied. Three patients were given Nilevar, 1 was given testosterone propionate and 6 were on a similar regime except that they received no hormone. A preliminary report on some of these patients including case records has been reported elsewhere.³

METHODS

The 10 patients 1 female and 6 male in this study were placed on the metabolic ward of the Department of Surgery. The operative procedures were considered comparable in magnitude. In 9 a partial gastrectomy was performed and in 1 a resection of the sigmoid colon (patient M M). They were weighed daily and all intake and output were accurately measured. Oral feeding in 3 patients consisted of accurately measured regular diets the food values for which were taken from standard diet tables. The other patients received a 6 feeding homogenized mixture of casein (Casec[®]) or milk a fat emulsion (Ediol[®]) dextrose and salts. The formula was designed to supply about 2000 calories and 0.2-0.3 gms nitrogen per kilogram of body weight and samples were analyzed for nitrogen fat sodium potassium water and total mineral content. After operation intravenous nutrients and

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Fig. 1 Body weight balance data and caloric intake of control patient C B F

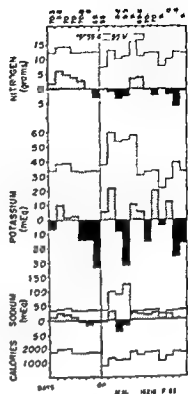


Fig. 2 Body weight balance data and caloric intake of patient M M who received Nilevar

water were adjusted so as to maintain the preoperative level of nitrogen electrolytes and where possible calories until oral feedings were resumed. Unfortunately, in several instances the caloric intake by intravenous feedings was lower. Nilevar and testosterone propionate were administered intramuscularly in doses of 25 or 50 mg daily beginning 1 to 3 days preoperatively. Chemical methods for analysis of formula and excreta have been described elsewhere.²

RESULTS

Two typical experiments are shown in Figures 1 and 2. Body weight, nitrogen, potassium, and sodium balances and the caloric intake are plotted daily on a patient (M M) who received Nilevar and on a control patient (C B F) who was on a similar regime but did not receive hormones. The negative nitrogen balance is definitely less in the Nilevar patient than in the control and the potassium balance parallels the nitrogen balance.

Table 1 is a summary of the results of weight and nitrogen balance changes on the 6 control patients, 3 patients receiving Nilevar, and the 1 patient receiving testosterone. All the patients were on an essentially similar regime. In all the cases except L F, G R, and N A, oral intake was very accurate because of the administration of the fluid formula described above as the sole oral feeding. In spite of the great care in measurement of both intake and output in this study, there were still sizeable daily fluctuations in nitrogen balance as noted in the 2 examples in Figures 1 and 2. The days of study were therefore divided into periods of 3 to 6 days, 1 period before operation and the other 2 after operation. The results in the table give the weight change and the nitrogen balance in kilograms and grams respectively per day in each period.

The previous physical status and dietary habits of the patient and ability

Table 1 *Weight Change and Nitrogen Balance in Surgical Patients with and without Hormones*

PATIENT SEX	AGE YRS	WT kg	PRIOR PERIOD			POSTOP PERIOD - 1			POSTOP PERIOD - 2					
			DAYS IN PTR	HORMONE (d)	WT Δ K/d	N BAL gm/d	DAYS IN PTR	HORMONE (d)	WT Δ K/d	N BAL gm/d	DAYS IN PTR	HORMONE (d)	WT Δ K/d	N BAL gm/d
CB-M	42	70	1	-	-0.1	-0.3	6	-	-0.3	-2.3	5	-	0	+1.1
GH-F	42	52	2	-	-0.1	+0.3	4	-	-0.5	-10.1	5	-	0	-1.0
CL-M	52	43	5	-	-0.1	-3.5	3	-	0	-9.0	1	-	-0.6	-1.6
GR-M	63	70	4	-	-0.1	+2.3	4	-	0	-0.2	4	-	-0.2	-2.0
LF-M	49	63	4	-	-0.2	+1.1	4	-	-0.3	-3.0	5	-	-0.1	-2
CB-F	37	57	2	-	0	+2.1	5	-	-0.1	-4.2	1	-	0	-3.9
AV					-0.1	+1.2			-0.2	-4.8			-0.2	-2.9
NA-M	50	67	6	N 1d	0	+1.7	4	N 1d	-0.4	0	3**	N 3d	-0.1	-7.0
MM-F	60	70	3	N 3d	-0.2	-0.1	4	N 1d	-0.3	-1.9	1	N 2d	+0.1	+0.9
ID-F	26	54	1	N 2d	+0.2	+6.0	4	N 3d	+0.2	+3.5	4	-	-0.1	-1.0
AV					0	+2.5			-0.2	+0.5			-0.1	-2.1
CK-M	42	54	2	T 2d	-0.7	+2.5	4	T 4d	-0.4	-0.2				

1 patient MM had a hemicolectomy. All the others had gastrectomy. Nilevar (N) and testosterone propionate (T) were given in doses of 2, m, 1 m daily except in the case of MM who received daily doses of 50 mg Nilevar d=day

**This patient (NA) had a febrile complication 4 days postoperatively

to adjust to the standard diet of approximately 2000 calories per day determined their preoperative nitrogen balances. Only 1 patient (L F) had a significant retention of nitrogen preoperatively possibly due to depletion due to the illness. The positive nitrogen balance in the other patients undoubtedly represented an adjustment to the diet. One patient (C I) was in a negative balance in the preoperative phase as well as following operation.

Although the amount of trauma was comparable in the group of patients in the series an individual variation in the response to the surgical procedure was to be expected. The average nitrogen balance in the first period after operation was -1.8 gms per day with a range from -0.2 to -10.1 gms per day in the 6 control patients. The average of the 3 patients receiving the anabolic hormone was -0.8 with a range of $+3.5$ to -2.5 gms per day and the patient receiving testosterone had a nitrogen balance of -0.2 gms per day.

Variable findings also characterized the second postoperative period. There was considerable difference between individuals in the time that it took to recover from the catabolic response to the procedure. One patient in the control group and 1 in the Nilevar group had reverted to a positive nitrogen balance by the end of 4 to 5 days whereas some of the other patients remained in markedly negative balance. Furthermore in 1 case (N A) a pulmonary complication on the fourth postoperative day resulted in delay of recovery and a markedly increased negative nitrogen balance in the second postoperative period.

On inspection of the average nitrogen balance in Table 1 the anabolic properties of Nilevar appear to be effective in the postoperative period. This effect exceeded the reduced negative nitrogen balance which occurred in the control patients in whom calories and nitrogen intake was maintained throughout the early postoperative period with intravenous infusions. Testosterone had a similar but less potent effect in the single case studied. However, the series is admittedly too short to give reliable statistical evidence of any difference between the effect of the 2 hormones or for that matter, to be definitive as to the effect of testosterone.

The administration of hormones to these patients did not appear to affect significantly the change of body weight following operation which may already have been minimized by the high nutrient intake in the postoperative period.

Although testosterone is known to cause salt and water retention the dose of Nilevar and in the 1 case testosterone given for the limited number of days in the present experiments resulted in no demonstrable sodium or water retention.

DISCUSSION

The administration of a new anabolic hormone Nilevar, in conjunction with a high caloric and nitrogen intake postoperatively has been shown to decrease the negative nitrogen balance due to operative stress. Testosterone propionate in the same doses seems to have a similar effect. It is probable that the mechanism of action of the 2 drugs is alike but this is yet to be proved.

In this study patients undergoing comparable operative stress were deliberately chosen to facilitate analysis of the results. It is not implied that

patients who are subjected to gastrectomy should receive such hormone therapy without more extensive study of the problem. It is more likely that patients in poor nutritive condition who must undergo severely traumatic surgery would benefit by a regime which would reduce the catabolism of surgery. Whether the hormones and a maintained high caloric and nitrogen intake will improve the postoperative period for such patients is still to be determined.

Preliminary RQ measurements³ and data from calculations of the components of weight change have shown that the anabolism due to Nidex occurs at the expense of fat which is apparently mobilized during the administration of the hormone. Whether this is a direct effect on fat or a secondary result due to the requirement of a source of energy for the synthesis of protein is yet to be determined. If the latter is the case, a high caloric source in the form of carbohydrate should spare fat during the administration of the hormone. The importance of maintaining caloric intake postoperatively has already been demonstrated in the previous work.¹

Theoretically it would seem that the prevention of protein breakdown should be beneficial in patients undergoing surgery. However it will take a much longer series of patients to prove that the regime described above hastens recovery or reduces postoperative complications.

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THE INFLUENCE OF GROWTH HORMONE ON PROTEIN METABOLISM*

MELVIN S. SCHWARTZ AND JOHN F. PRUDDEN

Protein metabolism in the normal person and in both the acute and convalescent burn patient has been studied with the aid of S^{35} -labeled methionine given intravenously in 100 microcurie doses. Sulfur labeled methionine was chosen since prior evidence had indicated that this amino acid plays an important role in wound healing processes¹; in addition the use of a labeled amino acid allowed for the synthesis *in vivo* of tracer protein native to the body.

The incorporation of isotopic sulfur into the globulin and albumin fractions of the plasma as well as the plasma levels of the non protein radio-sulfur were followed for an average period of 10 days after administration

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of the isotope. In certain of the normal and patient studies 50 mg of growth hormone* was injected intramuscularly beginning at 1 to 4 days following the methionine and continuing at 6 hr intervals until a total of 1000 mg had been given. Fifteen studies based on 6 patients and 2 normal individuals have been completed to the present time.

Blood samples are collected without anticoagulants, primarily to avoid introducing carrier sulfur impurities such as those found with heparin. This is critical since the analytical methods must necessarily be extremely sensitive to reflect accurately the low plasma levels encountered for the non protein sulfur and even for S^{35} albumin and S^{35} globulin etc. in the study. The method used is essentially that of Tarver² modified by Gray and Serralle. Involved are methanol precipitation of globulin, trichloroacetic acid precipitation of albumin and non protein sulfur precipitation by benzidine on the residual supernatant solutions from the latter two procedures. Final radioactive measurements have been made through β counting in a gas flow chamber.

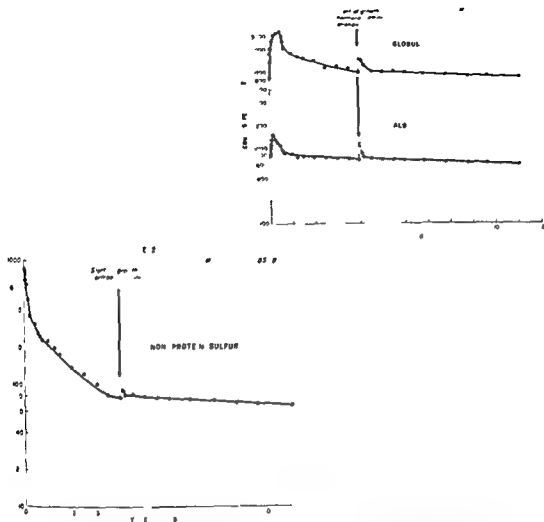
The curves representing the incorporation of radiosulfur into plasma albumin and globulin and the decline of non protein radiosulfur in the plasma are complex (See Figs 1 and 2). The peaks of the protein curves occur within the first 12 hours after injection of radioactive methionine; at the end of 24 to 36 hours the same semilogarithmic graph exhibits a tendency to fall away in almost linear fashion. Hypotheses about the metabolic pathways entered upon by methionine have been formulated in detail. It is reasonable to say that with or without growth hormone administration each protein curve is the resultant of at least two simultaneous processes—that of the incorporation of methionine into the protein (the *synthetic* phase) and that of the release of radiosulfur during the course of physiological turnover (the *degradation* phase). However the reincorporation of S^{35} , already synthesized and degraded once, into a second metabolic cycle—recutilization—complicates the analysis of these graphical results.

One simplification is possible. It is reasonable to assume that the tail end of such curves (the linear portion found beginning with the fourth or fifth day after injection) represents essentially but one of these two main processes, that of degradation. On this assumption the half lives of the degradation phase have been calculated from the straight line portions of the graphs. Table 1 lists the typical values found for globulin catabolism.

Table 1

PATIENT	TIME OF DETERMINATION POST BURN	HALF LIFE OF GLOBULIN CATABOLISM
AW	(with GH) 1 month post burn	34 days
	(with GH) 2 months post burn	97 days
	4 months post burn	125 days
FM	2 months post burn	60 days
	(with GH) 4 months post burn	150 days
RT	normal	140 days

*Growth hormone was supplied by The Armour Laboratories as Lot #R491025; it was free of vasopressor substances but was reported to contain 0.92 ± 0.098 units of thyrotropin per milligram.



It has been a reproducible finding that as convalescence from burn injury proceeds the half life for release of S^{35} from globulin lengthens. Degradation—and possibly true globulin turnover itself if the convalescent is approaching a steady state—slows down and approaches in absolute value that of the normal individual. It may be noted from Table I that the administration of growth hormone does not add any qualitative change to this general trend. Globulin has been used to illustrate the phenomenon; the albumin fraction of the plasma while also showing catabolic half time changes with convalescence behaves in a more complex fashion and its data are still undergoing study.

Growth hormone administration while not grossly influencing the catabolic phase of these protein curves does have a pronounced effect almost immediately post injection and on all of the substances followed—i.e. albumin, globulin and non protein sulfur (See Figs 1 and 2). Within 1 to 2 hours following the first administration of the hormone a pronounced rise or jump occurs in each curve studied. For albumin and globulin these jumps are evidence of sudden sharp increases in the rates and degrees of S^{35} incorporation into the proteins which may be interpreted as distinct anabolic boosts. These striking discontinuities in the graphs have been found in every instance of concomitant S^{35} and somatotrophic hormone administration.

The unique picture thus presented allows for 3 main possibilities (1) The rates of anabolism of albumin and globulin undergo absolute increases immediately after injection of growth hormone. However the presence of the same type of discontinuity in the curve of non protein sulfur (see Fig 2) suggests not *absolute* anabolic boosts but rather (2) a shift in the chemical equilibrium involved to increase the magnitude of the amino acid pool within the body, thus increasing the amino acid concentration in the plasma and in turn the synthesis of protein or alternatively, (3) alternatively a release of sulfur labeled amino acid alone or a release of both labeled amino acid and labeled protein into the plasma from storage in some organ or metabolic depot within the body, immediately after administration of growth hormone.

Liver perfusion studies have lent some confirmation to the latter two hypotheses. The speed with which the phenomenon follows upon hormone administration tends to favor the depot hypothesis and there is positive evidence that the isolated perfused liver can release plasma protein upon injection of growth hormone.³

It would seem from these preliminary results therefore, that growth hormone has at least a short term positive anabolic effect, the net result of which is to boost protein synthesis. This may result directly from increase of rates of anabolism but more probably occurs as a result of rapid mobilization from metabolic depots of stored protein and amino acid material. Such action produces a sudden break in the curves of sulfur incorporation into albumin and globulin similar to that which would occur with a second injection of non protein S³⁵ into the circulation. Indeed the curve of non protein sulfur shows evidence of such a reinjection. This phenomenon associated with growth hormone is receiving further study in our laboratory at the present time.

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AN IMPROVED METHOD OF MEASURING LOW PLASMA HEMOGLOBIN LEVELS AND SOME PRELIMINARY CLINICAL CONSIDERATIONS*

J. V. COCARLI AND H. A. NAUMANN

Interest in serum hemoglobin levels among surgeons has been mostly limited to conditions in which quite high levels occur such as with pump-oxygenators hemolytic transfusion reactions or in lower nephron nephrosis due to hemoglobinemia. These are usually measured by Hrm's method.¹

This investigation has been directed to develop a method of measurement and to study disease states with low abnormal plasma hemoglobin values i.e. between 2.5 and 10 mg per cent.

METHOD

Inhibitory Substance As shown by Creditor² and confirmed by Crosby and Furth,³ substances exist in plasma which inhibit the accurate measurement of hemoglobin by the quantitative benzidine reaction of Wu.⁴ These substances pose a special problem in dealing with low values as in our study.

It was found by one of us (H. A. N.) that in preparing the standard and blank the influences of these substances could be compensated for by adding the unknown plasma after prior deactivation of its hemoglobin by H_2O_2 . Thus unknown standard and the blank are all subject to the same interference.

Other Important Factors Hemolytic artefacts giving false high values of plasma hemoglobin may be caused by one or more of the following: prolonged application of venous tourniquet; skin antiseptic solution contaminating the needle; a small bore needle; water in the syringe; a poor venipuncture (important); roughness in handling the blood; improper anti-coagulant; too rapid centrifugation; the very slightest agitation of the red cell mass during pipetting (important); and the slightest contamination of the glassware (important). Only benzidine base and not the dihydrochloride is suitable as reagent.

Venipuncture and Plasma Separation A commercial heparin sodium solution 0.2 ml containing 1000 USP units per ml was measured under sterile conditions into a 20 ml syringe. Skin antiseptic was applied and the skin was dried thoroughly before venipuncture. The tourniquet was not applied until immediately before venipuncture. Any difficulty in entering the vein produced artefactitious hemolysis and free flow of blood into the syringe was essential. Eleven milliliters of blood were drawn gently into the syringe and mixed with the heparin. Ten milliliters of the blood were then slowly expelled along the wall of a graduated centrifuge tube which was centrifuged at 1000 rpm for 10 minutes. The supernatant plasma was aspirated by means of a pipette with attached rubber tubing without disturbing

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the packed cells in the least was then recentrifuged and pipetted off again leaving about 0.5 ml in the tube

Quantitative Benzidine Method Principle The sensitivity of the method was increased by the highest possible concentration in the reaction mixture of the most concentrated reagents and least diluted plasma. Recovery of hemoglobin was achieved by adding to the standard and blank solutions the same unknown plasma whose hemoglobin was deactivated by first adding H_2O_2 .

Benzidine reagent Ten grams of benzidine base (Harleco) were dissolved in 100 ml of glacial acetic acid at room temperature with occasional stirring. After centrifuging the reagent was clear yellow and was used for one day only.

Hydrogen peroxide Three per cent USP hydrogen peroxide was used undiluted and stored in the refrigerator.

Standard hemoglobin solutions A normal blood sample the hemoglobin content of which had been determined by the iron method (Sunderman *et al*⁵) was diluted 1:1000 with distilled water to give a stock solution of about 15 mg per cent which was stable for 3 weeks in the refrigerator. Working standards of 1, 2, 3 and 4 mg per cent were made fresh daily by appropriate dilution of small amounts of the stock standard with distilled water. The original blood could be frozen in quantities of 2 ml in test tubes and was stable for long periods.

Twenty per cent acetic acid Two hundred ml of acetic acid were diluted to 1000 ml with distilled water.

Procedure All glassware including syringes and needles were washed with 1:3 diluted concentrated H_2SO_4 , free of dichromate followed by 5 rinses with tap water, 3 rinses with distilled water and oven drying (avoiding rust from wire baskets).

One ml of plasma (if grossly hemolyzed and appearing brown to reddish brown prior dilution of 1:10 or more was necessary) obtained as described above was mixed with 1 ml of distilled water. This plasma dilution in amounts of 0.25 ml was pipetted into 5 test tubes labelled B (blank), 1, 2, 3 and 4 (mg per cent hemoglobin standard solutions) and 0.25 ml of H_2O_2 was added to each tube and mixed. While these mixtures were kept at room temperature for 10 minutes (deactivation of hemoglobin) another set of 5 tubes were labelled B, 1, 2, 3 and 4 and in this order 0.5 ml each of water, 1, 2, 3 and 4 mg per cent hemoglobin standard solutions diluted 1:2 were pipetted into the respective tubes. 0.5 ml of benzidine reagent was added to each of the 5 tubes and mixed. (Note: One set of standards must be run with each unknown plasma.) One tube labelled U (unknown) contained 0.25 ml of plasma dilution, 0.25 ml of water and 0.25 ml of benzidine reagent. Then 0.5 ml each of the benzidine mixtures B, 1, 2, 3 and 4 were pipetted into the corresponding tubes containing the deactivated plasma mixtures and 0.25 ml of H_2O_2 were added to the U tube with the unknown plasma. All solutions were carefully mixed with any drops adhering to the wall of the tubes. After 15 minutes 10 ml of 20 per cent acetic acid were added to each tube. The purple colored solutions were mixed and the optical density (logarithmic numerals) was read in a spectrophotometer at a wave length of 500 m μ using the blank to zero the instrument. We have used to advantage a Spectronic 20 (Bausch & Lomb) equipped with a Roto-Cell (A. H. Thomas).

Table 1 Scheme of Improved Benzidine Method

	PLASMA 1 2	H ₂ O ₂	H ₂ O	STANDARD BENZIDINE MIXTURES		H ₂ O ₂		20% ACETIC ACID
				STANDARDS	BENZIDINE			
UNKNOWN	0.25	—	0.25	—	0.25	0.25	15 MINUTES REACTION TIME	10.0
STANDARDS								
1 mg %	0.25	0.25	—	0.5 of 1 mg % 1 2	0.5	—	15 MINUTES REACTION TIME	10.0
2 mg %	0.25	0.25	—	0.5 of 2 mg % 1 2	0.5	—	15 MINUTES REACTION TIME	10.0
3 mg %	0.25	0.25	—	0.5 of 3 mg % 1 2	0.5	—	15 MINUTES REACTION TIME	10.0
4 mg %	0.25	0.25	—	0.5 of 4 mg % 1 2	0.5	—	15 MINUTES REACTION TIME	10.0
Blank	0.25	0.25	—	0.5 of H ₂ O	0.5	—	15 MINUTES REACTION TIME	10.0

Calculation The optical densities of the 4 hemoglobin standards were plotted on standard graph paper against the concentration in mg per cent. A graph was drawn and the value of the unknown read from the graph. Usually the graph was almost a straight line and the calculation was made by interpolation. This figure in mg per cent of plasma hemoglobin was corrected for the heparin dilution for variations in hematocrit adjusted for extra dilutions if any of plasma or for the final color solution.

DISCUSSION

Normal values By the use of the described method plasma hemoglobin values in normal individuals were found to range from 0.3 to 2.5 mg per cent with an average of 1.3 mg per cent.

Abnormal values Preliminary studies have shown the following abnormalities.

Blood transfusion without operation Bell⁶ in our University Hematology Laboratory using Hays' method reported elevations of plasma hemoglobin are quite common following transfusion. Our series in this field was small and showed minimal rises if any. Probably degrees of compatibility and age of the blood are important determining factors.

Table 2 Plasma Hemoglobin Levels After 500 cc Transfusion Without Operation

INDICATION FOR TRANSFUSION	BEFORE TRANSFUSION	IMMEDIATELY FOLLOWING TRANSFUSION	1ST DAY FOLLOWING TRANSFUSION
Secondary Anemia	11 Mg %	19 Mg %	1.0 Mg %
Secondary Anemia	14	22	0.8
Secondary Anemia	0.6	1.1	0.5
Secondary Anemia	0	1.1	0.7

Operation without blood transfusion Few operations of any magnitude are done these days without accompanying transfusion. The following were selected cases studied in which transfusion was either not performed or withheld until after the post operative specimen was drawn. They show definite but small degrees of hemoglobin elevation in two cases.

Table 3 Plasma Hemoglobin Levels in Operations Without Blood Transfusion

OPERATION	PRE OP	IMMED PO	1ST 10 DAY
Inguinal Herniorrhaphy	19 Mg %	06 Mg %	14 Mg %
Inguinal Herniorrhaphy	19	10	
Ventral Herniorrhaphy	11	13	14
Amputation of Toe Under Tourniquet (700 min for 20 min)	15	19	11
Ligation of Inf Vena Cava	10	31	13
Open Reduction of Fracture of Femur with Intra medullary Nail Fixation	21	13	16

Operation with blood transfusion Shinowara's first reported studies on a radical mastectomy receiving transfusion. Our studies also showed frequent rise above normal of values in patients undergoing major surgery requiring blood transfusions.

Table 4 Plasma Hemoglobin Levels in Operations with Blood Transfusions

OPERATION	CC OF BLOOD GIVEN	PRE OP	IMMED PO	1ST 10 DAY	2ND 10 DAY
Suprapubic Prostatectomy	1000	08 Mg %	31 Mg %	12 Mg %	
Intrapleural Thoracotomy with Plombage	2000	19	126	27	
Subtotal Gastrectomy	1500	23	194	110	14
Craniotomy with Resection of Temporal Lobe	4000	16	32	20	17
Transurethral Resection of Prostate	1500	12	40		23
Mitral Commissurotomy	1000	29	33*	25	
Suprapubic Prostatectomy	500	04	91	14	14

Drug influence No elevations were demonstrated after either inhalation or intravenous anesthesia nor after administration of the common intravenous fluids.

Disease states Abnormal levels (between 25 and 10 mg per cent) were demonstrated in patients with acute pancreatitis intra abdominal abscess

sickle cell anemia in remission polycythemia vera pseudomonas meningitis and in some patients with jaundice

SUMMARY

1 An improved method for measuring low plasma hemoglobin levels is described based on a new principle to achieve maximum hemoglobin recovery. Necessary precautions are presented to avoid hemolytic artefacts.

2 Beginning clinical studies are reported. 2.5 mg per cent has been found to be the upper limit of normal with our method. Elevated plasma hemoglobin levels have been demonstrated following operations with and without transfusion and in some disease states.

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INTRA AND EXTRACELLULAR CHANGES OF FLUID AND ELECTROLYTES IN EXPERIMENTAL UREMIA*

B F RUSH JR AND H T RANDALL

This study was designed to obtain a more complete evaluation of the shifts of fluid and electrolytes that occur in acute renal failure. Three general methods were employed: 1) measurements of extracellular fluid and electrolytes; 2) measurements of intracellular fluid and electrolytes in muscle biopsies; and 3) balance studies.

METHOD

Extracellular electrolytes were measured by standard methods previously described.¹ Plasma volume was measured by the use of Evans Blue dye (T 1824). Extracellular fluid volume was estimated by the diffusion of inulin and in 2 cases sucrose. Fluid spaces in the muscle biopsies were determined

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by the method of Mokotoff *et al*² using inulin in 5 cases and sucrose in 2. It is realized that the space defined by inulin or sucrose is at best, an approximation of true extracellular space. When we speak of extracellular space in this article it must be understood that we are actually referring to that compartment measured by inulin or sucrose.

Seven healthy mongrel dogs kept in an air conditioned kennel were used in these experiments. Control studies on each dog were first done. These consisted of a determination of the dogs weight, plasma volume, inulin or sucrose space, plasma electrolytes and muscle biopsies to measure the muscle extracellular and intracellular fluid and electrolytes. Following the control study, acute anuria was created in the animals by tying off the ureters at the brim of the pelvis transabdominally, under general anesthesia. The dogs were placed in metabolic cages and daily determination of plasma electrolytes, weight intake and output of water and in some cases electrolyte balance studies were done. The animals were maintained on nothing by mouth and it was planned to replace fluids intravenously in an amount approximately equal to 50 per cent of the daily weight loss, usually with a solution of 5 per cent dextrose and water. Sodium and chloride ions lost via the gastrointestinal tract through vomiting were also replaced. The volume of replacement varied more widely than planned but was always less than the daily weight loss.

After 72 hours of anuria all of the previous control studies were repeated. Determinations of the fluid volume and electrolyte content of the dead space in the renal pelvis and ureters in three animals revealed that loss of inulin or sucrose and electrolyte was not great enough to affect the study significantly.

RESULTS

On the regime of fluid replacement described above the dogs lost an average total of 7 per cent of their control weight (range 1 to 10 per cent) at the end of 3 days of anuria. Water balance studies revealed an average insensible loss of approximately 30 gm/kg/d.

In order to present the greatest amount of data in the most concise form

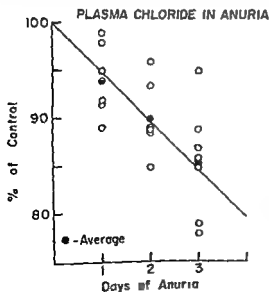
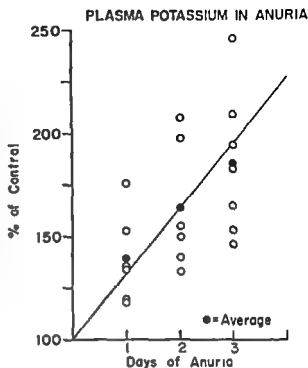


Fig 1. Change in plasma chloride in the course of 72 hours of anuria. There is a relatively consistent decrease each day in the plasma concentration. The average daily decrease can be plotted on an almost straight line. When one considers that the extracellular volume is also falling over this period it is evident that the total loss of chloride from the extracellular space is considerable.

Fig 2 Changes in plasma potassium concentration in the course of 72 hours of anuria. The rise in plasma potassium in the course of the regimen described was quite consistent with a straight line rise to a 100% increase in the 3 days. Since the total potassium in the extracellular space is relatively small it does not require the addition of much more potassium to accomplish this change. The consistent rise of potassium provides an interesting contrast to the changes seen in clinical material where this change is often variable.



the following charts are arranged in the form of scattergrams in terms of percentage of the control value the control value being set at 100 per cent. Figures 1 and 2 show the remarkably consistent changes of plasma concentration of potassium and chloride. Sodium (Fig 3) was somewhat variable with no significant average change. Average chloride concentration decreased 15 per cent. The changes recorded in extra and intracellular water and intracellular potassium and chloride are shown in Figure 4 and the significant features are pointed out by the legend. The most interesting correlation made here is that despite the fall in chloride concentration of the plasma the extracellular space as indicated by 3 separate methods decreased in volume. Intracellular chloride ion could not be presented adequately in this chart since the percentage increase was greater than the scale used. Control

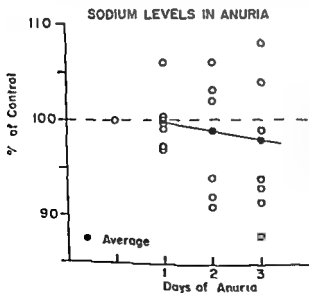


Fig 3 Changes in plasma sodium concentration in the course of 72 hours of anuria. Changes in plasma sodium were quite variable and the average concentration of sodium did not deviate from normal over the period of anuria.

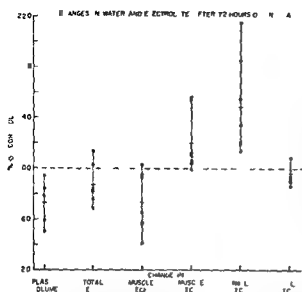


Fig. 1 Changes in intra and extracellular water and extracellular sodium and chloride. Extracellular water was decreased both in terms of plasma volume, total extracellular water and extracellular water in muscle biopsies. On the other hand, intracellular water in terms of the muscle biopsy findings increased an average of 20%. At the same time intracellular sodium increased and intracellular potassium decreased.

values in 5 dogs showed an intracellular content of chloride ranging from 7 to 10 mEq/L of muscle water. In 5 dogs after 3 days of anuria the intracellular chloride in muscle varied from 11 to 33 mEq/L of muscle water.

Chloride balance studies in 3 dogs showed no evidence of extrinsic loss which would explain the observed drop in plasma concentration (Table 1).

Table 1

DOG NO	CHLORIDE IN	CHLORIDE OUT	NET GAIN OR LOSS	OBSERVED LOSS IN ECF	UNACCOUNTED LOSS IN ECF
112	41.0	56.6	-15.6	-149	-165
139	97.2	118.2	-21.0	-51	-30
113	61.7	12.1	+49.6	-123	-173

Table 1. Chloride balance studies. Studies in these 3 dogs showed that the net extracorporeal loss of chloride could not account for the total amount of chloride lost from the extracellular fluid.

DISCUSSION

The findings indicate that after 3 days of anuria on the regimen described there is a loss of water, sodium and chloride from the extracellular space. Somewhat similar changes have been reported by others.^{3,4,5} This loss can not be fully explained on the basis of loss from the body. At the same time there is an increase of water, sodium and chloride in the intracellular space in muscle. Intracellular potassium decreases and extracellular potassium increases. These findings suggest that extracellular fluid and ions have shifted into the cells with a complementary shift of intracellular potassium and perhaps other ions out of the cell. There are many possible explanations for the changes observed and we will not deal with them here. On correlating the water balance data with the figures on intracellular water it is apparent that there has been no change or even an increase in total body water. This has occurred despite the loss of weight of the animals. Calculations of the water released from the tissues by the catabolism of fat and protein in the dogs over this period reveals that there is an adequate source for this water.

Some have maintained that inulin shifts into the cells in the course of uremia.⁶ If this were the case here the same apparent changes noted in

studies of potassium sodium and chloride in the muscle would be found, but at the same time there would be an apparent increase in extracellular fluid both in the total body studies and in the muscle biopsies. Also, the intracellular fluid in the muscle biopsies should appear to decrease—exactly the opposite of what was actually observed.

In these studies the dogs were given fluids sparingly in accord with present concepts of the treatment of acute renal failure. The changes found were however not at all characteristic of what one would expect in acute dehydration.

Some clinical and experimental studies of the fluid and ion changes in uremia have indicated an increase in extracellular water. A number of these studies have been based on calculations of the chloride space as an index of the extracellular space. If chloride shifts into cells during anuria such calculations would be most misleading.

SUMMARY AND CONCLUSIONS

Experimental renal failure was produced in 7 dogs by bilateral ureteral ligation. Prior to ligation observations of the weight, extracellular fluid and electrolytes and intracellular fluid and electrolytes of the dogs were done. During 3 days of anuria daily observations of plasma electrolytes, water balance, weight and in some cases chloride balance were done. At the end of 3 days of anuria the control studies were repeated. Results suggest that in the course of anuria there occurs an extracellular dehydration with a loss of fluid from the extracellular compartment. This change is accompanied by an increase in the cells of the extracellular ions sodium and chloride while some potassium appears to leave the cells.

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EFFICIENCY OF UTILIZATION OF ORAL FOODSTUFFS STUDIED BY CARBON DIOXIDE PRODUCTION*

JOHN M. KINNEY WILLIAM G. HAMMOND W. BANKS ANDERSON
AND ELLIOTT V. MILLER

With the growing recognition of the key role of available energy in modern surgery a wide variety of feeding mixtures and parenteral fluids is being marketed—with special emphasis on the provision of more and more calories per unit volume. This situation raises the need for a better understanding of the relationship between the rate of caloric supply and caloric utilization. Efficiency of foodstuff utilization can be approached by measuring new tissue laid down (using body composition studies, balance techniques, etc.) The alternative approach is to measure the amount of food not laid down as tissue and therefore undergoing combustion to yield a corresponding amount of heat along with the necessary utilization of oxygen and production of carbon dioxide.

A long term study of the factors influencing carbon dioxide production in surgical patients has been undertaken in this laboratory. One of these factors is the increased production associated with the ingestion of food termed specific dynamic action or SDA. The exact nature of the stimulus to this increased heat production or carbon dioxide output remains uncertain but there is reasonable agreement on certain facts.

SDA is a mild heat stress not associated with digestive processes and is unavailable as energy for muscle work. Some investigators have felt SDA to be a constant proportion of consumed calories (approximately 2 per cent for fat, 5 per cent for carbohydrate and 10 to 30 per cent for various amino acids). However, there is evidence that SDA may not be a biologic constant for a given food but rather may exhibit some variation depending on the condition of the patient and how the food is administered.¹ The energy loss due to SDA varies from 5 to 15 per cent on an average mixed diet and is insignificant in active healthy men. However, this loss may assume added significance in depleted, bed-ridden patients where tissues function less efficiently and positive caloric balance is difficult to maintain.

People probably differ in the hereditary control which they maintain over excess calories, namely whether the food is burned in the metabolic furnace or stored as carcass fat. Strains of mice have been shown to differ sharply in this oxidative tendency.² This would appear to have its clinical counterpart in the constitutionally slender people who somehow refuse to gain weight despite caloric intakes above their metabolic demands. People with endogenous obesity have been reported to have a reduced SDA,³ indicating a tendency to store rather than burn extra calories.

In brief, SDA appears to be the sum of all the intracellular handling charges associated with moving food products along various intermediary

*From the Department of Surgery, Harvard Medical School and the Peter Bent Brigham Hospital. We wish to acknowledge the support for these investigations from the U.S. Public Health Service.

We wish to express appreciation to Dr. F. D. Moore for his advice and encouragement to Mrs. Ruth Daniels for laboratory assistance and to Miss Hewa Schieve for diet management.

pathways some of which are associated with greater metabolic expense than others

There is little direct information regarding the effect of rate of administration of foodstuffs on SDA. There is some evidence that the larger the meal (which might represent a faster rate of administration) the larger the loss due to SDA. Lusk proposed that a plethora of digestion products in the tissues stimulated the heat increment of SDA⁴ and recent studies have offered support for this. The concept raises the possibility that slow rates of caloric administration might avoid most of the plethora and possibly lessen traffic along certain intermediary pathways which handle the overflow of digestion products and are associated with the extra carbon dioxide production. To explore this possibility and to obtain further information about patterns of carbon dioxide production in patients receiving special nutritional mixtures the following introductory study was undertaken.

METHOD

The studies presented here were performed with 6 normal subjects hospitalized on a surgical ward for a total of 40 days with measurements of carbon dioxide production being made at intervals of 45 minutes to 2 hours throughout each 24 hour period. The food was carefully standardized by a research dietitian to provide 47 per cent carbohydrate, 16 per cent protein and 36 per cent fat and was given in amounts of 1 600 cal, 2 400 cal, or 3 200 cal. Tube feedings were made by homogenizing and suspending the cooked food in a standard volume of water (usually 1 cc for each calorie). Tube feedings were given at uniform rates using a Bowman constant infusion pump connected to a plastic nasogastric tube. The carbon dioxide measurements were 7 minute collections performed as previously described.⁵ Analyses for residual carbon dioxide in the apparatus were performed with a Haldane instrument. Subjects had minimal physical activity during the study and were completely quiet in the supine position for 15 minutes or more before each run. Data were calculated as carbon dioxide production in $\text{cc}/\text{m}^2/\text{min}$ and the mixed diet as $\text{cal}/\text{m}^2/\text{hr}$.

Clinical Studies The carbon dioxide production of 4 subjects during a 24 hour fast and during the initial fasting hours of other studies provided a consistent pattern. The output decreased approximately 5 per cent over the first 4 hours after awakening and continued at this baseline level through the day and that night usually showing a low grade rise between 5 and 7 A.M. The following studies of carbon dioxide production are presented in terms of the stimulation to increases in carbon dioxide output above fasting levels associated with 3 normal meals and with the same food introduced as tube feeding at varying rates.

Four subjects were studied for 10 days when given normal meals and showed relatively marked increases in carbon dioxide output after each meal reaching 15 per cent to 20 per cent above baseline values with a variable tendency toward returning to baseline values between meals. The baseline is reached sometime between 3 and 5 hours after the evening meal and remains at this level until after breakfast the following morning.

In 2 patients who received the same food given as 8 equal meals over 12 hours the carbon dioxide output rose steadily over the period of the meals

EFFICIENCY OF UTILIZATION OF ORAL FOODSTUFFS STUDIED BY CARBON DIOXIDE PRODUCTION*

JOHN M. KINNEY, WILLIAM G. HAMMOND, W. BANKS ANDERSON
AND ELLIOTT V. MILLER

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nitrogen retention. Even here a negative balance on the first and second postoperative days cannot be avoided. The data of Kosteritz⁶ are impressive. He found that rats transferred from a stock diet to a protein free diet lost 16.2 per cent of their liver protein in 1 day and 23.8 per cent in 2 days. Subsequent daily losses were less. However, it is obvious that animals on depletion diets or patients unable to obtain protein rapidly lose nitrogen.

The present series of experiments shows that animals respond to decreased protein intake by reducing the rate of plasma protein turnover. No data are available on the size of the protein pools at the beginning and the end of the experiment although they probably would have dropped slightly. In this case the rates of synthesis and catabolism would be even slower than indicated in Table 1 since smaller pools would be involved. Peters⁷ has reported that the malnourished patient conserves nitrogen better than the normal individual.

The marked ability of depleted but otherwise normal dogs to double the

Table 1 Turnover rates for various experiments and the average daily percentage of turnover in the various groups

GROUP	TYPE EXPERIMENT	EXPERIMENT NUMBER	HALF LIFE IN DAYS	
			ALBUMIN	GLOBULIN
A	NORMAL ENDOGENOUS	A 1	13.0	8.9
		A 10	19.5	7.9
		A 6	13.0	8.7
		A 11	13.1	8.2
		A 12	12.6	8.0
		AVERAGE	14.2	8.2
		DAILY PERCENTAGE	4.9	8.4
B	NORMAL EXOGENOUS	A 13	10.2	6.6
		A 17	9.1	4.3
		A 18	10.0	5.5
		AVERAGE	9.8	5.5
		DAILY PERCENTAGE	7.1	12.6
C	DEPLETED ENDOGENOUS	A 16		14.7
		DAILY PERCENTAGE		4.7
D	DEPLETED EXOGENOUS	A 3	9.0	5.6
		A 14	11.5	6.9
		A 19	11.0	7.0
		AVERAGE	10.5	6.3
		DAILY PERCENTAGE	6.6	10.7
E	REFILETED ENDOGENOUS	A 20	6.6	5.9
		A 21	7.1	6.2
		AVERAGE	6.9	6.1
		DAILY PERCENTAGE	10.0	11.4

250 ml of blood was withdrawn from dogs A-6, A-11 and A-12 on the sixth day of the experiments. This did not change the turnover curves.

protein intake does affect the rate of plasma protein turnover. This effect is most pronounced in the previously protein starved animal.

METHOD

The details of plasma fractionation and the S^{35} assay procedure have been previously described.¹ The types of experiments are also similar and will be divided into 5 groups as shown in Table 1. The term endogenous refers to the protein synthesized by the animal after feeding the S^{35} labeled yeast. Exogenous refers to protein transfused in whole blood from a labeled donor dog. The Group A dogs were maintained on a stock diet and on day zero received a dose of S^{35} labeled yeast* mixed with dog meat. Blood samples of approximately 20 ml each were drawn periodically on succeeding days for about 3 weeks and the albumin and total globulin fractions were assayed for S^{35} specific activity as barium sulfate. Dogs in Group B, also maintained on a stock diet received a transfusion of 250 ml of labeled whole blood from donor dogs on day zero. During transfusion an equal volume of blood was drawn from a contralateral vein. Group C and D dogs were treated identically with those in Groups A and B respectively except that they had been maintained on the low protein diet of Weech *et al*² for periods of from 6 to 10 weeks prior to the beginning of the experiment. The Group E dogs had been on the low protein diet for 6 months. On the day prior to the start of the experiment a stock diet plus supplemental meat feedings was started.

RESULTS

The results of the various experiments including those previously reported are summarized in Table 1. The rates for all dogs within a group agree well except for the albumin in dog A 10. Such occasional variations have been noted by others.⁴ The percentage of daily turnover is derived from the graphically determined half life by the following expression:

$$\text{Percentage daily turnover} = \frac{\ln 2}{t_{1/2}} \times 100$$

If the group comparisons are made A against B and C against D it is seen that exogenous proteins turn over more rapidly than endogenous. This is true in both the albumin and globulin fractions for both normal and depleted animals although Group C contains only one determination.

Comparing Groups A against C and B against D a definite tendency is seen for a slower rate of turnover in protein depleted animals. With the repletion of a previously depleted dog a striking difference is noted. The turnover rates in Group E are considerably faster than the normals and the globulin turnover rate is more than twice as fast as that of the comparable depleted animal Group C.

DISCUSSION

The importance of protein metabolism in surgery has recently been stressed by Cole *et al*.⁵ Describing inadequate intake they state that "Without question this is the most common cause of hypoproteinemia." Their data on post surgical patients indicate that ambulation and a diet of basal plus 20 per cent consisting of 40 per cent protein achieves the maximum

*Obtained from the Division of Radioactive Pharmaceuticals, Abbott Laboratories

nitrogen retention. Even here a negative balance on the first and second postoperative days cannot be avoided. The data of Kosteritz⁶ are impressive: he found that rats transferred from a stock diet to a protein free diet lost 16.2 per cent of their liver protein in 1 day and 23.8 per cent in 2 days. Subsequent daily losses were less. However, it is obvious that animals on depletion diets or patients unable to obtain protein rapidly lose nitrogen.

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		AVERAGE	6.9	6.1
		DAILY PERCENTAGE	10.0	11.4

2.0 ml. of blood was withdrawn from dogs A-6, A-11 and A-12 on the sixth day of the experiments. This did not change the turnover curves.

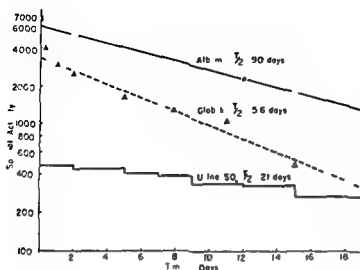


Fig. 1 Turnover curves for albumin, globulin and urinary SO_4 in a dog (A3) receiving a transfusion of labeled whole blood. Specific activity is plotted logarithmically against time.

rate of endogenous albumin turnover during repletion is significant. In these dogs the more rapid fall in specific activity results from increasing amounts of newly synthesized unlabeled albumin added to the circulation. Jeffay² has determined the hourly replacement rate of albumin in the rat on various protein diets. With inadequate diets the replacement rate was 3 to 4 mg/hr. with adequate diets it was 5 to 6 mg/hr. and with excessive diets it was 10 to 12 mg/hr. Previous data using nitrogen balance techniques showed protein conservation during times of protein depletion. The present data indicate that the rates of protein synthesis and breakdown (turnover) are decreased during protein depletion and increased during protein repletion. Thus the results of the balance studies are partially elucidated by the dynamic studies using the turnover method.

These data have been considered with a view of determining whether plasma proteins are converted directly to tissue protein without intermediation of free amino acids. Burke *et al.*⁸ have presented evidence supporting the view that direct conversion of parenterally administered plasma proteins to tissue proteins occurs. The low levels and the prolonged half life of excreted radioactive sulfur in relation to the injected proteins (Fig. 1) are compatible with the view of direct conversion of plasma proteins to more stable tissue protein. However the results are also compatible with the re-incorporation into tissue protein of free labeled amino acids resulting from the breakdown of the injected plasma proteins. In the previous discussion¹ this utilization of isotope was offered as an explanation for the apparently slower turnover rates of endogenous plasma proteins. Further experiments will be necessary to distinguish between these possibilities.

SUMMARY

1. Exogenous plasma proteins have an apparent turnover rate greater than endogenously produced plasma protein.
2. When a dog is starved in protein it turns over transfused and probably its own plasma proteins more slowly.
3. If dogs previously starved in protein are placed on a high protein diet there is a marked increase in the turnover rates of their endogenous plasma proteins.

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ACUTE DEPLETION OF GAMMA GLOBULIN IN THE DOG*

GEORGE L JORDAN JR OSCAR CREECH JR WILLIAM H BARNETT
ARIEL BARSELA AND MICHAEL E DEBARCA

Patients with agammaglobulinemia or extreme degrees of hypogammaglobulinemia fail to show an immune response to many stimuli. In such patients homotransplantation of skin may be performed successfully but it has not been possible to ascertain with certainty that successful homotransplantation results from the absence of gammaglobulin rather than from some other defect in the immune mechanism. A review of the literature failed to reveal reports of the experimental production of agammaglobulinemia in animals. However there are many agents which are known to affect the response to antigenic substances: cortisone and nitrogen mustard are two such agents. While neither has been reported to produce extreme degrees of hypogammaglobulinemia both appear to inhibit the production of antibodies.¹⁻⁴

This study was instituted in an attempt to produce a reversible agammaglobulinemia in the dog by acute depletion of gammaglobulin with exchange transfusions and inhibition of the production of gammaglobulin with cortisone and/or nitrogen mustard. The accomplishment of this objective would provide a valuable experimental tool for studies of the effects of hypogammaglobulinemia.

METHOD

Mongrel dogs were chosen as the experimental animal. Agammaglobulinemic blood was prepared by reconstituting washed canine red cells with agammaglobulinemic canine plasma (a by product of the commercial production of gammaglobulin for veterinary use) which contained an essentially normal distribution of other protein fractions and electrolytes.⁵ The serum levels of gammaglobulin were determined electrophoretically using the Tiselius apparatus and by the Kunkel test. Comparison of results of these 2 methods in our laboratory revealed good correlation, but in our hands the

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test was less sensitive than reported by others, so that one Kunkel unit represented approximately 100 mg per cent serum gammaglobulin and 10 units approximately 600 mg per cent. Normal Kunkel values ranged from 5 to 20 units in the animals studied.

To compare the effect of depletion of total gammaglobulin with that of an induced antibody response animals were immunized with typhoid vaccine to titers of 1:640 and the changes in typhoid titer determined, using a test tube technique.

Transfusions were performed by inserting polyethylene catheters into the femoral artery and vein under nembutal anesthesia. Two types of transfusion procedures were used. In the first type animals were bled from the femoral artery until bleeding ceased and the quantity of blood lost immediately replaced under pressure through the femoral vein catheter. This procedure required approximately 10 minutes. In the second type an estimated 25 per cent of the blood volume was removed through the femoral artery and this amount replaced by transfusion following which time aliquots of 15 or 20 ml of blood were alternately transfused and bled until a total of one to 1½ times the estimated blood volume had been exchanged. This procedure required 30 to 90 minutes.

Nineteen transfusions were completed in 11 untreated animals and 36 in 11 treated animals. In each experiment the serum level of gammaglobulin was determined before and 15 minutes following the completion of the exchange by one or both of the methods described above. Eleven of the transfusions in the untreated animals were of the first type described above including 3 in an immunized dog while 8 were slow exchanges. In 5 of the latter experiments Kunkel tests were performed daily in the post transfusion period to follow the return to pre transfusion levels.

Nineteen agammaglobulinemic transfusions were completed in animals treated with daily administration of 6 to 15 mg of cortisone per kg of body weight the drug being given at least 48 hours prior to the first transfusion and continued throughout the duration of the experiment. The return to pre transfusion levels of serum gammaglobulin was recorded by daily Kunkel tests in 8 experiments. Also 5 transfusions were performed in cortisone treated immunized animals and the immediate change in typhoid titer and the return to pre transfusion levels were recorded.

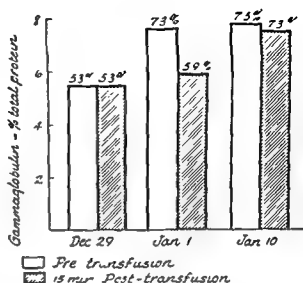
Four transfusions were performed 3 days after a single dose of nitrogen mustard of 25 mg or 5 mg per kg of body weight and in 3 experiments the return to normal levels recorded. Two additional experiments were performed in immunized animals.

Six transfusions were completed in animals treated with both cortisone and nitrogen mustard and the return to normal levels recorded following 4 of these. Two of these animals had received cortisone daily for 5 to 7 weeks prior to the administration of nitrogen mustard.

RESULTS

Three of the 4 untreated animals subjected to exsanguination and transfusion with agammaglobulinemic blood failed to show a significant decrease in the serum level of gammaglobulin at 15 minutes post transfusion and this type of transfusion also failed to reduce the typhoid titer in the immunized animal (Fig 1). The 1 animal which failed to maintain its pre transfusion

Fig 1 Failure of exsanguination and transfusion with agammaglobulinemic blood to significantly influence the serum level of gammaglobulin



level in the immediate post transfusion period died a few moments after completion of the experiment. Exchange transfusions by the slower method resulted in some depletion of gammaglobulin in each of 8 experiments but the return to pre transfusion levels was very rapid (Table 1)

Table 1 Acute Depletion of Gammaglobulin by Exchange Transfusion in an Untreated Animal

	TOTAL PROTEIN GRAMS %	GAMMAGLOBULIN % TOTAL PROTEIN	GAMMAGLOBULIN MG% %	GAMMAGLOBULIN KUNKEL UNITS
Pre transfusion	50	67	335	5.3
15 min Post transfusion	35	39	140	1.3

One transfusion of a treated animal usually produced a 50 per cent to 75 per cent reduction in the serum level of gammaglobulin and when transfusions were repeated daily a progressive lowering was recorded. In 1 cortisone treated animal complete agammaglobulinemia was recorded electrophoretically after 3 successive transfusions.

The average time required for the serum gammaglobulin level to return to that recorded prior to transfusion was longer in the animals treated with cortisone or nitrogen mustard than in the untreated animals and the combined effect of these 2 drugs was greater than that of either used alone. However the range in the treated animals overlapped to some extent that of the untreated group and no animal remained hypogammaglobulinemic for more than a few days except 1 animal treated with cortisone and nitrogen mustard that died on the fifth post transfusion day of pneumonia. On the day of death the serum gammaglobulin level was 1.4 kunkel units (Table 2).

Typhoid titers were reduced by exchange transfusion but returned to pre transfusion levels at a slower rate, average 13 days in the cortisone treated group and 9 days in the nitrogen mustard group. Transfusions of whole blood from non immunized animals containing a normal quantity of gammaglobulin but no detectable typhoid antibodies resulted in the same de

Table 2 Interval Required for Return of Gamma globulin to Pre transfusion Values Following Acute Depletion by Exchange Transfusion

GROUP		DAYS	
		RANGE	AVERAGE
A	Control	1.5	2
B	Treated		
	1 Cortisone	1.5	3
	2 Nitrogen mustard	2.7	4
	3 Cortisone and nitrogen mustard	4.11	7
	Total treated	1.15	5

crease in typhoid titer as that observed after transfusions with agammaglobulinemic blood and there was no difference in the rate of return to pre transfusion levels

The production of the hypoglobulinemic state appeared to serve as a stimulus to excess production of gammaglobulin as in many experiments the serum level rose higher than that recorded before transfusion (Fig 2)

DISCUSSION

The exsanguinating type of exchange resulted in an immediate replacement of approximately 80 per cent of the total blood volume with agammaglobulinemic blood. Therefore failure to significantly lower serum levels of gammaglobulin in this manner is confirmation of other studies leading to the conclusion that a large pool of extravascular protein is normally present which is in dynamic equilibrium with intravascular protein.¹ The decrease which followed the slower type of exchange is probably due partly to the larger volume of the exchange but in addition it is likely that the slower exchange allowed time for mobilization of extravascular reserves so that this pool was depleted also. Undoubtedly reduction to extreme levels of hypogammaglobulinemia could be accomplished by increasing the total volume of one exchange but multiple transfusions of limited quantity were tolerated better.

The fact that post transfusion values often exceeded pre transfusion values is considered evidence that the return to normal requires production of new

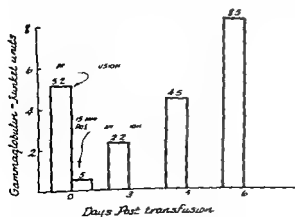


Fig 2 Rapid production of gamma globulin following acute depletion in animal treated with cortisone and nitrogen mustard

gamma globulin and that acute depletion acts as a major stimulus. Whereas cortisone and nitrogen mustard retard the synthesis of gamma globulin, their effect is limited so their use does not permit prolonged studies of the hypogammaglobulinemic state. However, the production of typhoid antibodies is much slower than the production of total gamma globulin and the titer may be kept at low levels by repeated exchange transfusions in treated animals.

SUMMARY

1. Acute depletion of gamma globulin in the dog can be accomplished by exchange transfusion; however, this animal has a large reserve store which can be mobilized rapidly and production of new gamma globulin occurs at a rapid rate.

2. In comparison to the results in untreated animals, cortisone and nitrogen mustard retard the return of serum gamma globulin to pretransfusion levels.

3. Typhoid antibodies are produced more slowly after acute depletion than is total gamma globulin.

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EXPERIMENTAL GRAFTING OF A SUSPENSION OF SKIN PARTICLES*

JOHN S. NAJARIAN AND H. J. MCCORKLE

The covering with epithelium of denuded areas resulting from the extensive loss of skin—particularly following severe thermal injuries—has long been a difficult surgical problem.¹ In such cases fresh and stored homografts have been used as a temporary epithelial covering.² More permanent results have been achieved with patch grafts³ or split thickness grafts removed at repeated intervals from the donor areas.⁴

The following experiments were performed in an effort to produce epithelial covering over widely denuded areas with the removal of relatively small amounts of skin from the available areas of intact skin.

From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco.

Supported by the Christine Breon Fund for Medical Research.

Table 2 Interval Required for Return of Gamma globulin to Pre transfusion Values Following Acute Depletion by Exchange Transfusion

CRITERION	DAYS	
	RANGE	AVERAGE
A Control	15	2
B Treated		
1 Cortisone	11	5
2 Nitrogen mustard	27	1
3 Cortisone and nitrogen mustard	111	7
Total treated	115	5

crease in typhoid titer as that observed after transfusions with gamma globulinemic blood and there was no difference in the rate of return to pre transfusion levels

The production of the hypoglobulinemic state appeared to serve as a stimulus to excess production of gammaglobulin as in many experiments the serum level rose higher than that recorded before transfusion (Fig 2)

DISCUSSION

The exsanguinating type of exchange resulted in an immediate replacement of approximately 80 per cent of the total blood volume with gamma globulinemic blood. Therefore failure to significantly lower serum levels of gammaglobulin in this manner is confirmation of other studies leading to the conclusion that a large pool of extravascular protein is normally present which is in dynamic equilibrium with intravascular protein.¹ The decrease which followed the slower type of exchange is probably due partly to the larger volume of the exchange but in addition it is likely that the slower exchange allowed time for mobilization of extravascular reserves so that this pool was depleted also. Undoubtedly reduction to extreme levels of hypogammaglobulinemia could be accomplished by increasing the total volume of one exchange but multiple transfusions of limited quantity were tolerated better.

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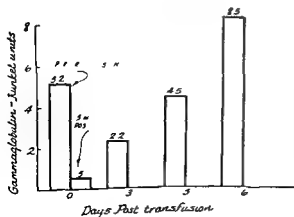


Fig 2 Rapid production of gamma globulin following acute depletion in animal treated with cortisone and nitrogen mustard

split thickness skin graft was obtained from the excised skin mounted on a block and divided into a suspension of skin particles as previously described. The fine mesh gauze with the film of skin particles was then placed directly over the fascia of the paravertebral muscles. The same type of dressing used for the animals in the first series was applied and left in place until the fourteenth day.

In group 3 similar full thickness skin defects of the same size were made on 4 rabbits. No skin grafts were applied but the dressings were the same as those used in groups 1 and 2. These animals were used as controls and also to observe the rate of contraction of such defects in the absence of skin grafts.

In all series of experiments the suspensions of skin particles were stained with Papanicolaou's stain for histological study of the size and nature of the divided skin particles.

RESULTS

Successful grafts were obtained in all but 3 of the 32 rabbits grafted. The 3 unsuccessful grafts resulted from technical errors in the preliminary experiments.

Inspection of the grafted site 2 weeks postoperatively revealed numerous scattered islands of epithelium (Fig 1a). These islands of epithelium through proliferation usually coalesced to completely cover the entire site by the third postoperative week (Fig 1b). The survival of grafts and rate of complete epithelialization was essentially the same whether they were applied to granulation tissue or directly on fascia.

Because the grafts were so thin, contraction was apparent in all instances. However, contraction of the grafts applied over granulation tissue was more



Fig 1 Photograph of rabbit's back showing (a) a recipient area 14 days after grafting the suspension of skin particles. Numerous scattered islets of proliferating epithelium are seen throughout the operative field. (b) Operative field 21 days after grafting the suspension of skin particles. The islets of epithelium have coalesced to form almost complete covering of the recipient area. (c) 5 weeks after a similar defect was made in another rabbit without applying a skin graft. There is almost complete obliteration of the defect with a stellate scar.

METHOD

Thirty six adult rabbits weighing from 5 to 7 pounds were used in this study. General anesthesia was produced with intravenous pentobarbital sodium solution and all procedures were performed with strict aseptic precautions. The suspension of skin particles was prepared with an electrical kitchen blender that has two component parts: a glass container and a base containing the electrical motor. The instrument functions by the action of 2 metal blades spinning rapidly on the bottom of the glass container. The glass container can be either autoclaved or cold sterilized with quarternary ammonium chlorides.

Preparation of the Grafts A split thickness skin graft was placed in the sterile container of the blender and enough cold normal saline was added to bring the skin graft into contact with the whirling blades. The minimum amount of normal saline added for this purpose was just sufficient to cover the blades. Lesser amounts were found to be ineffective in bringing the skin graft into contact with the blades. It was necessary to cool the saline to 0 to 10 C in order to dissipate the heat produced by the blender. The blender was operated at high speed for a period of 8 to 10 minutes with frequent observation of the temperature of the suspension throughout the procedure and addition of cold saline if the temperature rose above 37°C. The suspension of skin was then transferred from the container to a sterile beaker on the operating table. It was then applied with a sterile eye dropper or syringe to a piece of fine mesh gauze that had been previously cut to fit the area to be grafted. The fine mesh gauze acts as a filter removing the saline solution and allowing the skin particles to be deposited as a thin layer over one surface of the gauze. The gauze was then inverted and placed over the recipient area with the film of skin in contact with the area to be grafted.

Method of Transplantation Two series of experiments were conducted. In one the grafts were applied to freshly denuded fascia and in the others to granulation tissue. Comparison was made between the two groups with regard to the survival of the graft and the contraction that occurred.

In the first group an area of approximately 10 by 12 cm. of skin surface was excised down to the fascia overlying the paravertebral muscles from the backs of 12 rabbits. Fine mesh gauze impregnated with nitrofurazone ointment was placed directly over the defect. A dressing was applied using several thicknesses of surgical gauze held firmly in position with a thick piece of cotton cloth sutured to the skin margins at the periphery of the excised area. This dressing was changed after one week. At the end of the second week the dorsum of the ear was prepared as a donor site and a split thickness of skin approximately 2 x 4 cm. in size was removed with a skin grafting knife. This graft was then prepared as previously described and the fine mesh gauze with the film of skin particles was placed over the area of granulation. A similar dressing was used as before with the addition of a fine catheter incorporated into the surgical gauze. This catheter was used during the ensuing two weeks for the introduction of 20 to 30 cc. of normal saline solution daily to keep the dressing moist. The dressings were removed on the fourteenth postoperative day.

In the second group an area consisting of the full thickness of skin measuring not less than 8 by 10 cm. was excised from the backs of 20 rabbits. A

by this method would make its use undesirable in all but the most severe cases. However, if done successfully it would be an adequate temporary biological dressing for an extensively burned patient until definitive surgical repair could be done. The simplicity of this method of preparation and application of skin in the preliminary treatment of large denuded areas might be advantageous for use in isolated areas as well as in large medical centers.

SUMMARY

A method of preparation and grafting of a suspension of skin particles on rabbits is described. Ninety per cent successful grafts were obtained when they were applied by this method to either granulating wounds or directly on fascia. The possibility of the application of this method of skin grafting to human patients is suggested.

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DEVELOPMENT OF HISTOLOGICAL TECHNIQUES FOR THE DIFFERENTIAL STAINING OF BURNED AND NORMAL TISSUE*

J. RAYMOND HINSHAW AND HERMAN E. PEARSE

Much experimental work on burns has been hampered by the inability of an investigator to determine precisely the depth of injury produced. Comparisons of the effects of various exposure times and irradiances, comparisons of the ability of different agents to protect against thermal energy, and predictions of heat flow through tissue and thermal damage to tissue are dependent on the demonstration of the level at which destruction ceases and normal structures remain. The common histologic techniques for staining experimentally burned skin necessitate a highly subjective interpretation of the depth of injury. The investigator must be experienced in histology and able to distinguish between minute differences in cell composition and shape and two such experts do not always agree on the result.

A search was made for staining techniques which would yield a striking color contrast between normal and burned tissues. It was borne in mind that these methods, to be most useful, should be relatively simple and repro-

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marked than that observed when freshly denuded fascia was used as a recipient site. This difference in size may possibly be due to the added time factor of 2 weeks during which the inflammatory process associated with the formation of granulation tissue was developing. In the animals without grafts the defect contracted down to complete obliteration within 5 weeks leaving a stellate scar (Fig 1c).

Histological examination of the suspended skin particles revealed that the blender had divided the skin into clumps of epidermal cells, collagenous fibers, hair shafts, and hair follicles. The epidermal cell groups varied from 10 to about 100 cells. The 4 major types of epidermal cells, varying from the basal cells found in the lower malpighian layer to the nucleic cornified squamous cells, are represented in Figure 2. Proliferation of epidermis can be expected from the basal cells and hair follicles and some growth potential appears to be present in the precornified epidermal cells.

It seems possible that this method of grafting skin may be applied to patients with thermal injuries involving extensive loss of skin, and in whom the remaining skin is not sufficient to make suitable grafts by other means. The contracture of skin that may result from the extremely thin grafts applied

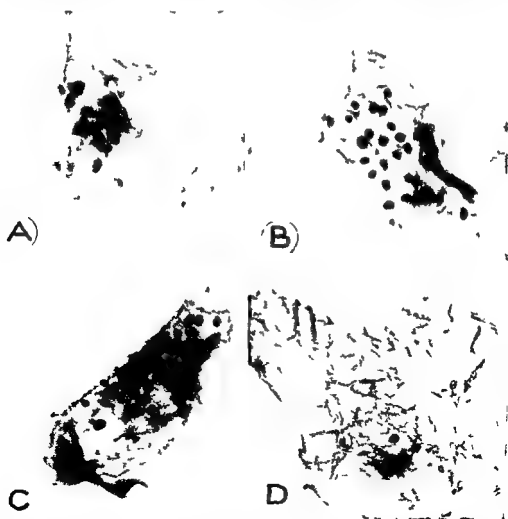


Fig 2 Photomicrographs of a suspension of rabbit skin particles, with representative groups of (a) basal (b) pre-cornified (c) follicular and (d) cornified epithelial cells.

Fig 1 Burned porcine skin stained with hematoxylin and eosin. Normal dermis on the right, burned dermis on the left. $\times 20$.



burned from normal tissues. This technique, a modification of Verhoeff's methods, stains the tissues with a mixture of hematoxylin, ferric chloride, iodine and potassium iodide. They are differentiated in aqueous ferric chloride and counterstained lightly with Van Gieson's stain. This produces a marked color difference between burned and normal tissues (Figs 2 and 3) and the results are so consistent that duplicate staining is not necessary. Normal epidermis is gray, burned epidermis is yellow. Normal dermal collagen is yellow and burned collagen is black or purple.

DISCUSSION

In many experiments with burns it is necessary to determine as accurately as possible the depth of injury resulting from a particular thermal insult. In this laboratory, specimens of more than 3000 burns have been stained with hematoxylin and eosin. With this technique, if staining conditions are well

Fig 2 A section from the same block of tissue shown in Fig 1 but stained by the method described in this paper. Note the marked contrast between burned (black) dermis on the left and normal (light) dermis on the right. $\times 20$.

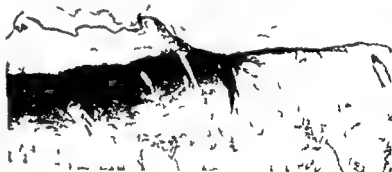


Fig 3 A partial thickness burn produced by a 0.5 second exposure to radiant thermal energy. Stained by the method described. $\times 150$.

ducible in any tissue laboratory and that workers with little training in micropathology should be able to interpret the results without difficulty. Such techniques have been found and their reliability and accuracy have been demonstrated in well over a thousand experimental burns. One of the most useful methods is described and illustrated here; others will be reported subsequently.

METHOD

The experimental animals were young Chester White pigs anesthetized with intraperitoneal Dialin Urethane (Ciba) in doses of 70 mg/kg of body weight. The hair was removed with electric clippers and the skin was gently washed. A heat source consisting of a modified 21 inch Army carbon arc searchlight² produced burns which varied from mild erythema to severe full thickness damage. The port which controlled the size of the burn was 1.7 cm in diameter. Exposure times from 0.3 to 30.0 sec and irradiances from 32 cal/cm²/sec to 0.16 cal/cm²/sec were used.

Eighteen to 24 hours after the burns were placed biopsies were taken across the injured areas so that normal tissue was included on each side of the burn. The biopsies were fixed in 10 per cent formalin or Bouin's solution and embedded in paraffin.

Various staining solutions were selected for trial because results from their use in standard histologic techniques led us to believe they might aid in the differential staining of burned skin. When a technique offered promising results staining and differentiating times were altered to achieve ideal staining conditions. Sections were then examined by both experienced and inexperienced microscopists to determine what color combinations could be interpreted most easily and most accurately. The final step consisted of altering staining, differentiating, washing and counterstaining times so that the desired end result could be reached with large groups of slides and without individual attention to each slide.

Staining Method. Fix tissues in 10 per cent formalin and embed in paraffin. To prepare the staining solution with the aid of heat dissolve 1 g hematoxylin (CC) in 20 ml absolute alcohol. Filter and add 8 ml of a 10 per cent aqueous solution of FeCl₃ and 8 ml of an iodine solution made by dissolving 2 g iodine and 1 g KI in 100 ml of distilled water.

Remove paraffin from the sections in the usual manner. Immerse the sections in the above staining solution for 20 to 30 min. Differentiate in a 2 per cent aqueous solution of FeCl₃. A rack containing about 25 slides can be dipped up and down in this solution 20 times or so in 20 to 30 sec. Wash immediately and thoroughly in fresh tap water. Place in 95 per cent alcohol for 1 min. Wash in running tap water thoroughly for 5 min. Counterstain for 15 sec in Van Gieson's stain. Dip slide once in tap water. Dehydrate in 95 per cent alcohol followed by absolute alcohol. Clear in xylene and mount.

These precautions should be observed. The staining solution should be used within 24 hr and a fresh solution should be substituted after each 75 slides. Differentiation in 2 per cent aqueous FeCl₃ is accomplished more rapidly than in the standard Verhoeff stain. Until an exact time is established a single slide may be examined in water to determine the degree of differentiation. Depending on the result the slides may be either restained or differentiated further. The last washing in tap water must be thorough. A short counterstaining time with Van Gieson's stain yields slides which may be easily interpreted. The more brilliant colors produced by a longer counterstaining time are less easy to read.

RESULTS

The result of the use of 1 of the 5 methods which have been developed will illustrate what can be expected of a stain in the differentiation of

Corticoid Changes During and Following Operation

THE DISTRIBUTION OF CORTICOSTEROIDS IN THE PLASMA AND RED CELLS OF SURGICAL PATIENTS*

ARNOLD MITTLMAN AND HAROLD G. BARKER

The partition of corticosteroids between red cells and plasma of human blood is controversial but most investigators determine only concentration in plasma since plasma is a less troublesome medium to extract. Simpson and Tait¹ have added radioactive compound F and aldosterone to whole blood and found roughly 25 per cent of these materials in the red cells. Hecter *et al*² have found significant quantities of added steroid in the red cells of perfusing media. On the other hand Morris *et al*³ and Nelson and Samuels⁴ have found no adrenocortical steroids in the red cells of peripheral and adrenal vein blood. We have measured this partition in normal human blood and have also measured the effects upon it of diurnal variation and of operation.

METHOD

In the determination of the normal partition we utilized a total of 2 liters of blood, each portion of which was freshly drawn and immediately processed as described below. The measurement of diurnal variation was carried out on 4 preoperative surgical patients from each of whom a 100 cc blood sample was drawn both at 8 A.M. and 5 P.M. The study of the effects of surgery was conducted in the same 4 patients (3 herniorrhaphies and 1 cholecystectomy). Again, 100 cc blood samples were utilized and these were drawn at 8 hr., 24 hr. and 72 hr. after operation. All bloods were drawn using heparin as the anticoagulant and centrifuged promptly (within 10 min.) in a water cooled Sorvall centrifuge at 8000 rpm and the plasma promptly separated. The cells were then resuspended and separated twice using physiological saline solution in quantities equal to that of the original plasma. The aqueous fractions were added to the plasma and the red cells were lysed with an equal volume of distilled water.

The plasma and lysed red cell samples were then extracted by the alcohol precipitation method of Bongiovanni.⁵ To insure completeness of extraction 2 warm alcohol and 1 butanol wash of the precipitated blood proteins were carried out and the combined extracts evaporated to dryness by nitrogen vacuum distillation in a 45°C. water bath. The dried extracts were suspended in distilled water, hydrolyzed with β -glucuronidase (ketodase) and subjected to continuous dialysis and extraction by the method of Lombardo *et al*.⁶ The resulting extracts were chromatogrammed for 72 hr. with appropriate standards in toluene-propylene glycol at 30°C. The strip areas corre-

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controlled there is a color difference between acidophilic burned epidermis and basophilic normal epidermis. However, when many slides are stained simultaneously, even this difference is not consistent. Mallory's stain and Masson's trichrome stain are of equally limited value. With all 3 techniques one must determine the extent of injury by the size and shape of nuclei and by almost insignificant differences in the composition and color of collagen. This is very time consuming and it is disconcerting how often experienced microscopists disagree on the findings.

Because the method presented here and other methods to be reported subsequently provide such a marked color difference between normal and burned tissues the slides can be read easily and rapidly. The accuracy of the methods has been checked by staining sections from the same blocks of tissue by a number of techniques. Long careful examination of the hematoxylin and eosin sections and a much more rapid examination of the specially stained sections lead to virtually the same estimate of the depth of damage.

The method reported here has already proved invaluable in experimental work on the effects of superimposing two radiant energy thermal burns³ on the use of flash creams in protecting bare skin against radiant thermal energy¹ and on the prediction of the depth of injury by surface appearance of burns.⁴

SUMMARY

1. A search was made for histologic techniques which would provide a striking color contrast between normal and burned tissues.

2. One of the most useful techniques is described and illustrated.

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less this is done there is risk of steroid entering the cells *in vitro*. Since transfer in the opposite direction is unlikely it is advisable to wash the cells and preserve the wash solution for addition to the plasma prior to processing. In this manner one recovers the trapped plasma and increases the yield permitting more accurate analyses for a given volume of whole blood drawn.

Experimental work involving the determination of steroid levels following the administration of exogenous steroids is probably best done on whole blood rather than plasma alone. Recently we have given 300 mg of hydrocortisone hemisuccinate intravenously to a patient and thereafter determined the plasma red cell partition on freshly drawn blood. A considerable amount of material having the same running rate as compound F was found in the cells. Likewise it is probably wise to use whole blood analyses when ever *in vitro* experiments are being done or if prompt separation of samples drawn from patients is not possible.

SUMMARY

The plasma red cell partition of compound F and tetrahydro compounds has been determined in freshly drawn human blood under varying conditions. The steroid was found to exist largely in the plasma fraction (although small amounts were found in the red cells) in normal resting individuals and also during the fluctuations in whole blood steroids which resulted from both diurnal variation and the operative stress reaction. When exogenous steroids are administered however large quantities enter the red cell and under these conditions it is advisable to use whole blood for analysis. The same is true whenever the cells remain in contact with the plasma for considerable periods *in vitro* such as when prompt separation is impractical or in the conduct of perfusion experiments.

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Table 1 - Diurnal Variation in Blood Partition of Steroids Mean Values in 4 Patients*

TIME	PLASMA		RBC	
	COMB. I	TETRAHYDRO	COMB. I	TETRAHYDRO
A.M.	27	35	6	9
P.M.	18	17	3	4

*Micrograms percent

sponding to compound I and the tetrahydro compounds were eluted and measured by the BTZ reaction (micro adaptation)⁷ and the Porter Silber reaction is modified by Bongiovanni using a Coleman Universal Spectrophotometer.

RESULTS

The 21 of freshly processed whole blood from normal humans showed compound I concentrations of 27.5 μ g per cent in the plasma and 8.6 in the red cell portion. The tetrahydro compound concentrations were 35 in the plasma and 11.2 in the cellular portion. In the study of diurnal variation the mean values for the 4 unoperated patients showed an evening drop in compound I and tetrahydro compound concentrations in both the plasma and red cell portions (See Table 1). The study of the effect of the combined stress of anesthesia and operation showed that the mean values in the 4 patients rose well above the normal at 8 hr and thereafter began a gradual decline but at 72 hr the figures were still above normal. The changes were reflected in the concentrations of both compound I and tetrahydro compounds in both plasma and red cell fractions (See Table 2).

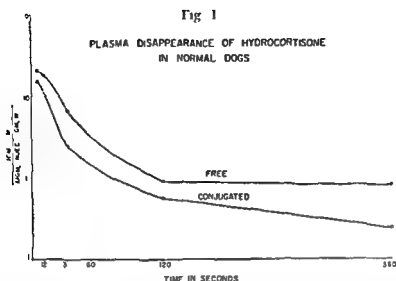
Table 2 - Postoperative Blood Partition of Steroids Mean Values in 4 Patients*

TIME	PLASMA		RBC	
	COMB. I	TETRAHYDRO	COMB. I	TETRAHYDRO
Preop	See Table 1			
8 hr postop	55	67	11	13
24	48	54	7	7
72	40	45	6	7

*Micrograms percent

DISCUSSION

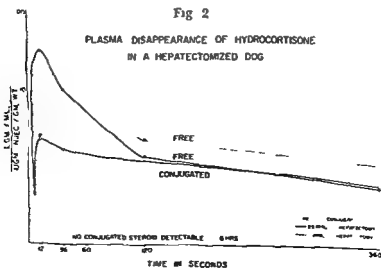
It seems evident from our studies that endogenously produced adrenocortical steroids circulate in the blood largely in the plasma fraction although small amounts do enter the red cells. Furthermore fluctuations in total blood steroids with both diurnal variation and with the stimulation of operative stress are well reflected by the concentrations in plasma. Thus in conducting experimental work which deals only with endogenous steroids it is probably justified to continue the practice of limiting analyses to the plasma fraction. This is particularly true if the separation of plasma from cells is carried out promptly after the samples are drawn. There is evidence that in



plasma than from any other vein in the body indicating some extrahepatic conjugation. Since this was not the anticipated finding the plasma was tested for completeness of extraction. It was demonstrated that all of the chloroform extractable hydrocortisone was removed from the plasma prior to B glucuronidase extraction.

In Figure 1 is shown a composite semilog plot from 5 normal dogs of the plasma disappearance and conjugation curves of instantaneously injected hydrocortisone. The values obtained from the 5 animals were made comparable by expressing the concentrations as $\mu\text{g/ml} / \mu\text{g}$ injected per gram of body weight. The concentration of the conjugated steroid was extremely high in the first pass arterial sample indicating very rapid conjugation of hydrocortisone. The simultaneous mixing and disappearance of hydrocortisone and its conjugation curve is not well enough established at the present time to derive the number of exponential components.

The plasma disappearance and conjugation curves of hydrocortisone in a dog 25 min following hepatectomy and 6 hr following hepatectomy are illustrated in Figure 2. The experiment demonstrates impaired steroid conjugation when no liver tissue is present. However 6 hr following hepatec-



A PRELIMINARY STUDY OF THE DYNAMICS OF STEROID CONJUGATION*

M. DON TURNER, JAMES D. HARDY, AND JOHN O. DAMMITER, JR.

The chemical and physicochemical events that occur following the elaboration of the steroid hormones from the adrenal cortex are slowly being revealed. However, the alterations which occur in these steroid hormones as they exert their influence upon the physiologic mechanisms of the organism and the actual nature of their action remain to be solved.

It is known that the principal glucocorticoid, hydrocortisone, is secreted by the adrenal cortex into the circulation as the steroid alcohol. A large part of the total hydrocortisone is readily converted to the tetrahydro form and excreted by the kidney as a glucuronide conjugate, and a smaller portion as a sulfate conjugate. It appears that the conjugation process represents an event or series of events which inactivates the steroid hormone and prepares it for rapid renal excretion. The selective renal excretion of the conjugated form of the steroids is well established. It is possible that the study of events leading up to conjugation will yield considerable information concerning the form of the steroid hormone at the instant of action.

This paper is a preliminary report on a study of the dynamics of steroid conjugation.

METHOD

The method used for the determination of free and conjugated hydrocortisone blood levels were those of Nelson & Samuels¹ and of Bongiovanni.

Adrenal vein blood and analysis. Blood samples were obtained directly from the adrenal veins of 5 patients during suitable surgical procedures. These adrenal vein blood samples were analyzed for the free alcohol of hydrocortisone and for the glucuronidate forms.

Plasma disappearance of free hydrocortisone and the appearance of conjugated hydrocortisone. Five milligrams of hydrocortisone was suddenly introduced into the venous circulation of 5 normal dogs. Arterial samples were obtained at 6, 12, 36, 60, 120, 360 sec. and the plasma analyzed for both free and conjugated steroid.

Conjugation of hydrocortisone in the totally hepatectomized dog. A series of dogs was totally hepatectomized and the early plasma disappearance and conjugation curves of hydrocortisone were constructed for these animals at different time intervals following hepatectomy. The curves were determined at 25 min., 1 hr., 3 hr., and 6 hr. following hepatectomy.

RESULTS

The human adrenal vein and systemic plasma levels of free hydrocortisone and of the glucuronidate form were measured in 5 patients. The concentration of the conjugated hydrocortisone was much higher in adrenal vein

Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi. This work was performed under Army Contract No. DA 49-007 MD G-27, Office of the Surgeon General, Department of the Army.

With the technical assistance of Thomas Carter and Jennice Cooper.

working hypothesis that the biological half life of hydrocortisone is approximately 3 hr in the dog

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STEROID METABOLISM IN MAN MINUTE HYDROCORTISONE OUTPUT OF LEFT ADRENAL *

Preliminary Report

JAMES D HARDY AND M DON TURNER

One of the requirements for successful surgery is that the patient have adequate adrenocortical function and multiple studies have indicated that surgery usually increases corticoid production. Evidence has been derived by various means the more direct being the measurement of blood corticoid levels and the daily urinary excretion of steroids in human beings. Hume and Nelson¹ actually measured hydrocortisone production in dogs by cannulating the adrenal vein following contralateral adrenalectomy. However so far as we are aware no studies of the steroid content of adrenal vein blood have previously been reported in the human. Thus the purpose of this investigation has been to measure hydrocortisone concentration in adrenal vein blood samples taken at operation and by means of timed adrenal vein blood flow to effect an approximation for the output of hydrocortisone per minute. The concentrations of both the free and conjugated forms of the steroid have been measured in peripheral and adrenal venous blood samples taken simultaneously.

METHOD

Anatomic Considerations The venous drainage of the left adrenal gland courses largely through the central vein to enter the left renal vein approximately opposite the left ovarian vein in the female and the left spermatic vein in the male. This vessel is usually almost 2.5 cm in length and from 2 to 4 mm in diameter as it emerges from the adrenal gland. By inserting a No. 17 needle into the adrenal vein and temporarily occluding the vessel as it enters the renal vein the blood flow through the former can be collected over a timed interval. To be sure there exist patients in whom the adrenal is drained by several other smaller veins in some of whom the flow by alter-

Department of Surgery University of Mississippi Medical Center Jackson Mississippi
The work was performed under Contract No. DA-49-007 MD-627 Office of the Surgeon General Department of the Army
With the technical assistance of Thelma Carter and Virginia Ward

tomy no conjugated steroid could be detected in the plasma after the intra venous introduction of the free form. This has been demonstrated out to 6 hr in 2 dogs at the present time. The conjugation mechanism loses most of its capacity immediately following hepatectomy and continues to decline to 1 hr when the conjugation process can no longer be detected.

DISCUSSION

With the present method for the determination of the glucuronide conjugate of hydrocortisone² we obtained 1 peak of the conjugated form almost immediately following rapid injection of the alcohol of hydrocortisone. If hydrocortisone is infused slowly over a period of approximately 15 min we obtain a peak of the conjugated form 1 hr from the beginning of the infusion. This latter finding has been confirmed by a number of investigators. The early conjugation peak was first thought to be an artefact. Many attempts by the authors to disprove it have failed so far. We are at the present time attempting to determine if the early peak conjugate is the tetrahydro form and if it is actually bound to the glucuronides.

If the existence of this rapid conjugation peak is established it will throw some light on the dynamics of hydrocortisone in the circulation. First of all the data indicate that the substance responsible for the conjugation mechanism is elaborated into the circulation by the normal liver. For this reason conjugation proceeds not only in the liver but throughout the circulation. Therefore conjugation of the steroid may be considered in part at least an extracellular process. If it is true that any portion but not all of the hydrocortisone released by the adrenal cortex into the blood is instantly conjugated this indicates an enzymatic reaction which can approach completion only as the more soluble steroid conjugates are excreted by the kidney. Since no conjugates were found 6 hr following hepatectomy even after the administration of 5 mg of hydrocortisone it is assumed that the turnover time of hydrocortisone is less than 6 hr in the dog. This latter assumption represents a working hypothesis and certainly no definite conclusion can be drawn at the present time.

The present data support the idea that the conjugation process acts as a safety mechanism to buffer the action of excess steroid and to enable the kidney to readily eliminate excess steroid by increasing the solubility of these materials in aqueous solutions.

CONCLUSIONS

1. The conjugation of hydrocortisone with glucuronides occurs very rapidly. A large portion of the hydrocortisone secreted by the adrenal gland is apparently conjugated at the instant of its release into the circulation. Therefore at least a part of steroid conjugation occurs in the circulation and not in the liver.

2. The presence of a normal liver is necessary for the conjugation process to continue. However immediately following liver removal steroid conjugation still proceeds but at a rapidly declining rate until after 6 hr no conjugation occurs.

3. Six hours following hepatectomy no glucuronide conjugate of hydrocortisone could be detected even after the administration of 5 mg of the hydrocortisone alcohol. On the basis of this data the authors have set up a

working hypothesis that the biological half life of hydrocortisone is approximately 3 hr. in the dog

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METHOD

Anatomic Considerations The venous drainage of the left adrenal gland courses largely through the central vein to enter the left renal vein approximately opposite the left ovarian vein in the female and the left spermatic vein in the male. This vessel is usually almost 2.5 cm. in length and from 2 to 4 mm. in diameter as it emerges from the adrenal gland. By inserting a No. 17 needle into the adrenal vein and temporarily occluding the vessel as it enters the renal vein the blood flow through the former can be collected over a timed interval. To be sure there exist patients in whom the adrenal is drained by several other smaller veins in some of whom the flow by alter

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With the technical assistance of Thelma Carter and Virginia Ward.

nate channels may be considerable. Furthermore, it may happen that the suction exerted on the needle introduced into the adrenal vein is such as to collapse the vein and thus to decrease the rate of flow per minute; in this case the blood that is obtained is relatively low in volume but high in corticoid content reflecting the fact that adrenocortical secretion is in this respect independent of the rate of blood flow.

The adrenal gland itself is approached through the gastocolic ligament. The left kidney and the tail of the pancreas are first identified, and the posterior parietal peritoneum overlying the pancreas is divided along the inferior border of the body and tail of the pancreas which is gently elevated and retracted cephalad with a Deaver retractor. This course of dissection permits exposure of the left renal vein, the superior mesenteric vein, and the splenic vein. Moreover, by palpation in the space between the aorta and the superior pole of the left kidney, the left adrenal gland can be identified. When the adrenal and the left renal vein have been adequately exposed, the relatively large central adrenal vein is readily visualized and isolated.

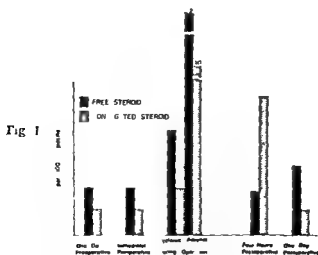
The values for the concentrations of free and conjugated hydrocortisone in systemic portal and adrenal venous blood were determined by the methods of Nelson and Simuels¹ and of Bongiovanni.² There was no significant difference between the concentrations in portal and those in systemic blood samples.

RESULTS

To date the analyses are complete in 10 patients and the values in a representative subject are shown in Figure 1. It may be seen that there was a prompt rise in both free and conjugated forms during operative stress followed by a decline after 1 hr. At the time when the systemic venous plasma levels of corticoids were 25.6 and 11.6 gamma/100 ml for free and conjugated forms respectively, the corresponding concentration for free hydrocortisone in adrenal vein blood was 312 gamma and for conjugated forms 90 gamma.

In the 10 patients the average measured adrenal vein blood flow was 25 cc/min; the average adrenal venous plasma level of free hydrocortisone was

SYSTEMIC AND ADRENAL PLASMA HYDROCORTISONE LEVELS



224 gamma per cent and the average adrenal venous plasma level of conjugated hydrocortisone was 125 gamma. In systemic blood the average preoperative values for free and conjugated forms were 5.6 and 7.8 gamma/100 ml (8 patients) 21.0 and 16.7 gamma/100 ml respectively during operation (16 patients) and 9.9 and 22.2 gamma/100 ml 1 hr after the close of the operation (7 patients). The relative preponderance of conjugated forms postoperatively perhaps reflected the rapid conjugation of the large amount of free hydrocortisone secreted by the adrenals during operation. As a rule the rise in plasma corticoid levels occasioned by operation had subsided by the end of from 24 to 48 hr.

Estimation of Hydrocortisone Output Per Minute. Since the concentration of free hydrocortisone in systemic blood during operation averaged 21.0 gamma and that in adrenal venous blood 224 gamma/100 ml of plasma the adrenal would appear to have effected an increase of 200 gamma per 100 cc of plasma flowing through it. Since the average plasma flow per minute was approximately 12.5 cc (total blood flow 25 cc) the output of free hydrocortisone per minute by the left adrenal may have been one eighth of 200 gamma or 25 gamma.

The minute output of conjugated hydrocortisone as reflected in adrenal venous blood was estimated in a similar manner. At the time when the plasma concentration of conjugated hydrocortisone in systemic venous blood was 16.7 gamma/100 ml that in adrenal blood was 125 gamma a difference of 108.5 gamma. If the adrenal plasma flow per minute was 12.5 cc the secretion of conjugated forms per minute would appear to have been on the order of 13.6 gamma. Although it appears that the adrenal is elaborating a conjugated form it is more probable that rapid conjugation occurs as the steroid enters the circulation.

In brief on the basis of these data it is estimated that both adrenals contributed during operation a total of approximately 77 gamma of hydrocortisone per minute to the plasma red cell content not considered. Furthermore if the subject whose adrenal vein plasma flow was the greatest (56 cc/min) were to be considered separately the hydrocortisone secretion per minute was on the order of 63.8 gamma free and 46.5 conjugated (Total = 110.3 gamma). It would appear that the selective increase of free as compared to conjugated hydrocortisone in systemic blood during operation is due to the rapid release of large quantities of the free alcohol by the stressed adrenals.

It is also estimated from the plasma data that the total hydrocortisone output per 24 hr is 34 mg. If one includes red cell hydrocortisone content estimated at 25 per cent of the total blood content by some workers then the output per 24 hours might be 47 mg.

SUMMARY

1. The effect of operation upon the systemic plasma levels of both free and conjugated hydrocortisone have been given.
2. By means of blood samples taken from the left adrenal vein at laparotomy an approximation for hydrocortisone secretion per minute has been made.
3. During operative stress in 10 patients the average total hydrocortisone secretion was estimated at 77 gamma per minute. In 1 patient who exhibited

the highest adrenal vein blood flow measured the value was on the order of 110 gamma per minute

4 The total hydrocortisone production in the non stressed subject is estimated at 47 mg /24 hr

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THE EFFECT OF STIMULATION OF THE FEMORAL NERVE ON ADRENAL 17 HYDROXYCORTICOSTEROID SECRETION IN DOGS*

RICHARD H EGDahl AND JOHN B RICHARDS

It has been suggested that nervous stimuli constitute an important if not crucial factor in the production of adrenocortical stimulation following trauma Long¹ has demonstrated adrenal ascorbic acid and cholesterol depletion following electrical stimulation of the central portions of exposed sciatic and brachial nerves in rats Hume and Wittenstein² have shown in dogs and Gordon³ in rats that the application of certain stimuli (fracture operative trauma) to a limb results in adrenocortical stimulation and that this response may be prevented by denervating the limb

In the foregoing studies indirect indices of adrenocortical secretory activity were employed The present investigation was undertaken to study adrenocortical function following stimulation of the femoral nerve in dogs utilizing a technique which provides a specific and direct index of the secretory activity of the adrenal cortex and permits frequent sequential determinations in the same animal In addition the effect of induced high blood concentrations of 17 hydroxycorticosteroids upon the adrenocortical response to nerve excitation was studied

METHOD

Ten male mongrel dogs weighing 13 to 19 kg were used in this study The dogs were anesthetized with intravenous pentobarbital sodium and the right lumboadrenal vein of each animal was cannulated according to a technique described by Hume and Nelson⁴ This preparation permits intermittent collection of venous blood from the cannulated adrenal gland The dogs were allowed to recover for a period of 36 to 48 hr after which they were re-

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anesthetized with intravenous pentobarbital sodium and a femoral nerve in each dog was isolated and severed. A period of 30 to 60 min was allowed to elapse before the collection of control adrenal vein blood samples was begun. All adrenal blood samples were obtained by collecting the total venous effluent of the right adrenal gland for a period of one minute. Following the collection of control blood samples the positive and negative leads from a 6 volt dry cell battery (Burgess 11H) were placed on the surface of the isolated and exposed nerve for periods of 1 or 5 min. The nerve became progressively charred during this procedure. Adrenal venous blood samples were collected at frequent intervals following stimulation of the nerve. In 5 of the 10 dogs the peripheral end of the severed nerve was stimulated and adrenal venous blood samples were obtained prior to stimulation of the central end of the nerve. In the remaining 5 animals only the central portion of the severed nerve was stimulated. In the first 3 dogs continuous mean arterial pressures were obtained throughout the experiment from a femoral arterial cannula connected to a strain gauge and recording potentiometer.

In 3 dogs following the stimulation of the central end of the severed femoral nerve and collection of adrenal venous blood samples an intravenous infusion of 17 hydroxycorticosterone (Hydrocortone 100 mgm/1000 ml of 0.85 per cent sodium chloride solution 40 drops/min) was administered for a period of 1 hr. During the first 30 min of hormone infusion a number of adrenal venous blood samples were collected and at frequent intervals for the remaining 30 min. Samples of femoral venous blood were obtained immediately prior to the hormone infusion period, at the time of nerve stimulation and at the termination of the infusion. All blood samples were analyzed for 17 hydroxycorticosteroid content by the method of Nelson and Samuels⁵.

RESULTS

Table 1 lists the adrenal 17 hydroxycorticosteroid output values obtained following femoral nerve stimulation in 7 dogs. It can be seen that in the animals tested (Dogs 1, 2, 3, 4, 7) no significant change in adrenal 17 hydroxycorticosteroid secretion occurred following stimulation of the peripheral portion of the transected femoral nerve. Stimulation of the central end of the nerve, however, resulted in a marked increase in adrenocortical secretory activity in all dogs. This response was almost immediate with maximal 17 hydroxycorticosteroid output occurring within 5 min after the period of nerve excitation in 7 of 9 experiments. The adrenocortical stimulation was of a transient nature and in most cases adrenal corticoid secretion reverted to control levels within 30 min. No significant change in adrenal venous blood flow was noted. In 3 animals continuous mean arterial pressures were obtained during the experiments. The mean arterial pressures in animals 3, 4 and 7 prior to nerve stimulation were 100, 105 and 115 mm Hg respectively. Femoral nerve stimulation was not accompanied by more than 5 mm Hg variations in mean arterial pressure in these dogs.

Table 2 lists the results obtained from 3 dogs in which the effect of increased peripheral blood concentration of 17 hydroxycorticosteroids upon the adrenocortical response to nerve stimulation was studied. In all 3 animals a marked increase in adrenal corticoid secretion was elicited by stimulation of the central portion of the femoral nerve despite the high plasma

Table 1 The Effect of Stimulation of the Femoral Nerve on Adrenal 17 Hydroxycorticosteroid Secretion in Dogs

PERIOD OR STIMULATION	NO.	NO.	Ave $\mu\text{g}/\text{min}$	ADRENAL VENOUS BLOOD 17 HYDROXYCORTICOSTEROID SECRETION $\mu\text{g}/\text{min}$									
				0-5	5-10	10-20	20-30	0-5	5-10	10-15	15-20	20-30	30-60
1	1	1	$0.5 \pm 0.08^*$	0.2	0.3	2.8	1.8	11.1	11.6		11.2	9.9	
2	1	1	0.1 ± 0.1	0.0†	0.1	1.0	0.6	11.2	1.2	1.8	3.1	0.0	
3	1	1	0.1 ± 0.2	0.1	0.6	0.1	0.0	3.7	16.0	1.1	10.2	10.2	0.8
4	1	3	1.9 ± 0.7	1.1	0.3	2.2	0.6	12.2	3.2	1.6	0.0	1.6	1.1
	3	1	1.2 ± 0.7					10.6	6.2	0.0	0.1	2.6	0.5
5	1	2	3.0 ± 1.8					3.0	27.0	16.6	6.6	1.6	3.2
	3	1	3.1 ± 0.2					33.8	27.6	20.2	28.6	3.1	3.2
6	3	3	0.3 ± 0.1					31.1	32.8	2.8	2.8	9.6	3.0
7	3	3	1.5 ± 1.7	0.8	2.2	0.1	0.0	12.3	9.1	2.0	1.7	7.1	3.1

Standard Deviation

†Zero Numbers Represent Steroid Output Values Below Sensitivity of the Analytical Method (0.1–0.3 μg)

Table 2 Effect of Stimulation of the Iliac Nerve on Adrenal 17 Hydroxycorticosteroid Secretion in Dogs with Increased Peripheral Blood Concentrations of 17 Hydroxycorticosterone Period of Nerve Stimulation—one minute

ADRENAL VENOUS BLOOD 17 HYDROXYCORTICOSTEROID SECRETION $\mu\text{g}/\text{min}$																					
PERIOD OF 17 HYDROXYCORTICOSTEROID INFUSION																					
CONTROLS		CONTROLS										MINUTES AFTER NERVE STIMULATION									
DOG NO	NO	\bar{x} $\mu\text{g}/\text{min}$	2	5	8	10	15	20	30	NO	\bar{x} $\mu\text{g}/\text{min}$	2	5	8	10	15	20	30			
8	1	$1.5 \pm 1.6^*$	0.8	0.8	10.4	10.8	7.2	2.6	0.1 (5.0)†	3	2.6 ± 0.3 (34.0)	14.8	14.2	10.8	3.8	3.2	1.2	2.6 (37.0)			
9	1	0.9 ± 0.5	0.6	4.6	14.6	9.1	3.4	0.7	2.2 (5.0)	1	2.2 ± 0.6 (82.0)	5.6	8.8	7.1	3.8	1.0	3.9	4.9 (114.0)			
10	3	3.3 ± 2.7	0.8	1.2	4.0	4.0	1.8	1.0	0.2 (1.6)	3	3.1 ± 1.4 (60.0)	5.8	7.5	8.8	13.0	18.6	20.0	3.8			

* Standard Deviation

† Numbers enclosed by parentheses represent plasma concentrations of 17 hydroxycorticosteroids ($\mu\text{g}/100$ ml plasma) in peripheral blood

concentrations of 17 hydroxycorticosteroids in the circulating blood stream. Furthermore in 2 animals (8 and 9) the same pattern and magnitude of adrenocortical response to nerve stimulation were observed in the periods prior to and during hormone infusion. In animal 10 no appreciable increase in adrenal steroid output occurred following the initial nerve stimulation but a marked increase resulted from the second stimulation (during the hormone infusion period). This result might possibly have been due to improper electrode placement at the time of the initial stimulation.

DISCUSSION

The principal steroid hormone secreted by the adrenal cortex of dogs is 17 hydroxycorticosterone.^{11, 12} The quantitative estimation of 17 hydroxycorticosteroids in adrenal venous blood of this animal (as performed in these studies) provides a direct and specific index of adrenal cortical secretory activity.

Previous studies of adrenal cortical function following nerve stimulation include the additional variables and likely pituitary-adrenal stimuli of increased blood pressure, alterations of neural reflexes, and changes in respiration. These physiologic changes are accompaniments of electrical stimulation of a nerve. Fortunately we are not limited to electrical nerve stimulation for thermal, chemical, and physical methods have been successfully used.¹³ Especially interesting is the work of Grinnit and Lundberg¹⁴ who found that thermal stimulation of the central end of peripheral nerves in cats resulted in selective small fiber stimulation. The stimulus used in the present study was most likely of a thermal nature for the nerve became progressively charred and warmed during the application of direct current. An early direct electrical effect cannot be ruled out. In the present study, two facts stand out clearly: 1) The stimulus was definitely effective; 2) It did not result in changes of blood pressure or respiratory movement. Because of the absence of these other physiological phenomena, it is felt that the method of nerve stimulation utilized was particularly satisfactory. Although analysis of potentials coming from the nerve would be of great interest following this stimulation, and delineation of thermal, chemical, and electrical components of the stimulation mechanism would be desirable, such data are not necessary to the conclusion that the stimulus as used resulted in a greatly increased adrenal cortical secretion without the blood pressure changes attendant upon electrical stimulation. It is probable that such specific stimulatory effect was achieved because fewer and perhaps smaller nerve fibers were stimulated.

The adrenal cortical response following stimulation of the central end of the femoral nerve is marked but transient. It has been found that the response to thermal stimulation of nerves rapidly deteriorates.¹⁵ After the adrenal response has subsided, there is an immediate return to high levels with further central nerve stimulation. In at least 1 animal, the 5 min stimulation resulted in a greater adrenal secretion than after 1 min.

Stimulation of the peripheral end of the femoral nerve did not result in adrenal cortical stimulation in any instance. Gordon³ has postulated that the mechanism of adrenal cortical stimulation following scalding is a release of a humoral factor at the sight of the scald which in turn activates the pituitary-adrenal system. The lack of adrenal response following stimulation of

the peripheral segment of a severed nerve indicated that a local humoral agent capable of eliciting pituitary adrenal stimulation is not produced as a result of peripheral nerve stimulation in general. This does not rule out, of course, the possibility of central nerve stimulation leading to the release of a central nervous system neurohumor which causes release of pituitary ACTH. Indeed this is most likely.¹⁰

It has been suggested by Sayers and Sayers¹¹ that ACTH release is effected by the level of circulating adrenal cortical hormones and that as the blood level of such hormones decreases as a result of peripheral utilization more ACTH is released. In the present study central nerve stimulation was performed in 3 dogs with induced high peripheral blood levels of 17 hydroxycorticosteroids. A marked adrenal cortical stimulatory effect was observed following central nerve stimulation. It is apparent that an increased blood level of adrenal steroid hormones does not inhibit the adrenal cortical response to peripheral nerve stimulation.

SUMMARY

Stimulation of the central end of a transected femoral nerve in anesthetized dogs results in a transient but marked increase in adrenal 17 hydroxycorticosteroid secretion. A similar stimulation of the peripheral portion of the severed femoral nerve produced no detectable change in adrenal corticoid secretion. The pattern and magnitude of adrenocortical response to neural stimulation are not altered by the presence of increased concentrations of 17 hydroxycorticosteroids in the circulating blood stream. These experiments indicate that neural excitation may play a role in the production of the pituitary adrenal stimulation observed following trauma.

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A COMPARISON OF THE STEROID FORMATION BY INCUBATED ADRENAL SLICES OF HYPERTENSIVE PATIENTS AND NORMOTENSIVE PATIENTS*

D. Y. COOPER, J. ROBERTS, J. C. TOUCHSTONE AND O. ROSENTHAL

Two years ago we presented to the College of Surgeons a method for the quantitative and qualitative assay of *in vitro* steroid formation by adrenal tissue from human patients. Since this report, adrenals from 16 patients adrenalectomized for the treatment of hypertension and from 6 patients operated upon for palliation of carcinoma of the breast or prostate have been analyzed. The number of cases appears to be sufficiently large for a preliminary statistical comparison of the 2 groups. In addition adrenal tissue from 4 normotensive cancer free patients biopsied because of suspected adrenal disease was studied.

METHOD

The technique described in the previous paper has been employed throughout this study¹ and therefore will be described only briefly here.

Adrenal glands were obtained at the first stage operation from patients with hypertension and cancer of the breast and prostate. Biopsy samples were taken in patients who had had no recent operation. This tissue was iced immediately and taken to a cold room at 4°C where the incubates were prepared. After removing the peridrenal fat the adrenal gland was weighed. A portion of the gland was saved for pathologic study. The remainder was cut into thin slices by means of the Stadie Riggs slicer. Approximately 10 gm. of these slices was placed into a flask containing 10 cc. of the patient's plasma drawn prior to operation. Streptomycin and penicillin were added to prevent bacterial growth. The flasks were then immersed into a constant temperature water bath at 37.5°C and incubated for 24 hr. while a slow current of 95 per cent oxygen 5 per cent carbon dioxide passed continuously through the vessels. After completion of the incubation the tissue and plasma were homogenized with a glass homogenizer and the proteins precipitated with acetone.

After removal of the precipitated proteins by filtration, the acetone was removed by distillation *in vacuo*. The aqueous residue was extracted with ethyl acetate and chloroform. After washing the extract with dilute sodium hydroxide and water the organic phase was evaporated to dryness by distillation *in vacuo*.

The residue was chromatographed on filter paper by the technique of Burton, Alfaro and Keutmann.² A scheme of the procedure is shown in figure 1.

The strips were read at 245 m μ in the Beckman spectrophotometer using an adaptor described by Tennent, Whitla and Florey.³ Representative strips

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With the technical assistance of Maria Konowickaja.

The human adrenal tissue was supplied by Doctors W. S. Blakemore, W. F. Lums Jr., H. A. Zintel, John Murphy and Paul Lieberman.

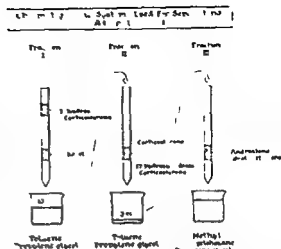


Fig 1 Scheme of the chromatographic separation of adrenal steroids. Fraction I contains the Before F F and F (a ketol) regions. Fraction II contains the B and S region. Fraction III contains the Δ^4 androstene 11β O 1 17 androstene 11β 9 17 dione and some of the B and S steroid that might run over from Fraction II.

were sprayed with blue tetrazolium dye and read at 600 m μ in the Beckm in spectrophotometer.

Absorption curves for all strips were obtained by plotting the optical density of each point against its distance from the starting line of the chromatogram. Five definite areas or peaks resulted. The first area noted after the starting line runs a little slower than Compound 1 and has been labelled before F area since the steroids present in this position have not been positively identified. The second peak contains hydrocortisone (cortisol) which has been positively identified. The third region studied is that in which Compound E is known to separate in this system. Several steroid substances are present in this region but none of these materials has been identified. This region has been labelled the E position. The fourth peak is in fraction II and is the area in which known Compounds B and S would separate in this system. Analysis of this position indicates that not only Compounds B and S are present but also additional unidentified steroid substances. The fifth area contains Δ^4 androstene 11β O 1 17 androstene 11β 9 17 dione which has been positively identified in this system from extracts of human incubates.⁴

Estimates of the quantity of steroid represented by each peak have been made by measuring the area under the curve of each ultraviolet peak and comparing this area with that produced by a standard amount of 11-deoxy corticosterone. The values are expressed as μ g of steroid per gram of adrenal tissue. The results are summarized in Table 1.

Comparison of Steroid Formation by Adrenal Slices of Carcinoma and Hypertensive Patients. The chromatograms of the adrenal incubates from both groups of patients were identical with reference to number and position of absorption maxima seen. Differences in the individual compounds that form the 5 areas studied may exist since of all the steroid compounds present only 2 have been positively identified.

It is evident from Table 1 that there were striking differences between the groups in the quantity of steroids formed in these 5 positions. The largest difference occurred in the B and S and 11β hydroxy androstenedione position. The steroid formation in tissue from hypertensive patients averaged 198 and 209 μ g/gm in the B and S and the 11β hydroxy positions respectively while corresponding means for the carcinoma patients were only 78 and 82 μ g/gm.

Table 1 Steroid Formation ($\mu\text{g/gm}$) by Incubated Human Adrenal Slices

Mean Values \pm Standard Error
Number of Experiments in Parenthesis

WEIGHT	NO ACTH				JO ACTH					
	BEFORE F	F	Γ	B+S	11 β hyd	BEFORE F	F	E	B+S	11 β hyd
Normal	— (4)	226 (4)	118 (4)	90 (2)	82 (2)	116 (2)	38, (2)	181 (2)	—	—
Carcinoma	4.90 \pm 21 (6)	192 \pm 29 (6)	61 \pm 16 (2)	78 \pm 19 (5)	82 \pm 13 (2)	140 \pm 30 (2)	138 \pm 32 (2)	91 \pm 12 (2)	162 \pm 37 (4)	171 \pm 33 (4)
Hypertension	22 \pm 40 (16)	224 \pm 26 (16)	140† \pm 10 (15)	198† \pm 20 (2)	209† \pm 22 (7)	171 \pm 19 (14)	109† \pm 9 (14)	210† \pm 33 (11)	213* \pm 50 (7)	318 \pm 93 (4)

*Significantly different from carcinoma group ($p < 0.5$)

†Significantly different from carcinoma group ($p < 0.1$)

A less striking but significant finding was the approximately 50 per cent increase in formation of the steroids of the before F and E positions by adrenal incubates from hypertensives. The mean steroid formation in these two positions averaged $42 \mu\text{g/gm}$ and $76 \mu\text{g/gm}$ greater than that in the incubates from cancer patients.

The small difference between the mean values of cortisol formation by the incubates from carcinoma and hypertensive patients resulted from the great variability of cortisol formation by incubates of hypertensive adrenals. Values for cortisol formation by these hypertensive adrenal slices varied from $150 \mu\text{g/gm}$ to $400 \mu\text{g/gm}$.

Further study of the distribution of hydrocortisone data reveals that the cortisol formation of hypertensive adrenals falls predominantly into two definite groups: a low group forming 100 to $200 \mu\text{g/gm}$ of Compound F and a high group with values ranging from 300 to $400 \mu\text{g/gm}$. This finding indicates that the hypertensive adrenals are not drawn from a homogeneous population as will be discussed later on.

Comparison of Hypertensive and Cancer Cases with Normotensive Cancer-free Cases In view of the small number of patients available in this group no statistical analysis has been carried out on these data. Steroid formation by the incubates of this group resembled that of the cancer patients with the single exception of the steroid formation in the E position which was apparently greater.

Effect of ACTH on the Incubates of Hypertensive and Cancer Patients The addition of 5 units of ACTH to the incubates resulted in 2 changes in the general pattern of steroid formation by these adrenal incubates. No increase in formation above that found without ACTH was noted in the B and S position of hypertensive incubates. The average values of 16 hypertensive incubates for B and S steroid formation were $198 \mu\text{g/gm}$ before ACTH and $213 \mu\text{g/gm}$ after ACTH. A significant increase of from $78 \mu\text{g/gm}$ to $198 \mu\text{g/gm}$ was found in the carcinoma incubates. In contrast stimulation of the E position in the incubates from carcinoma patients was small. Addition of 5 units of ACTH only increased the E position formation from $61 \mu\text{g/gm}$ to $91 \mu\text{g/gm}$ which was not significant statistically.

Increases of approximately 50 per cent were noted in the other positions after the addition of ACTH as shown in Table I.

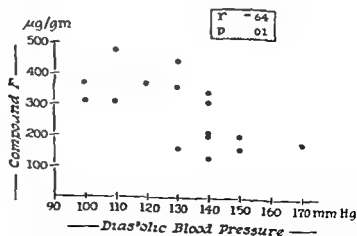


Fig 2 The relationship between compound F formation in $\mu\text{g/gm}$ and diastolic blood pressure

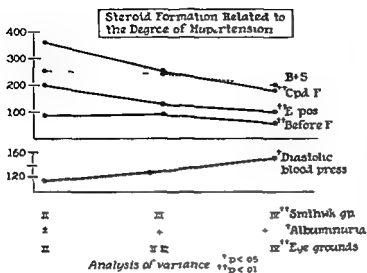


Fig 3 Relationship of formation of Compound F and of the steroids in the Before F E and B and S regions to increasing severity of hypertension in 12 patients. Statistical significance was computed by the analysis of variance.

Relationship Between Steroid Formation in Adrenal Incubates and Clinical Hypertension In an attempt to explain the separation of the cortisol formation into 2 definite groups the correlation of diastolic blood pressure to cortisone formation was studied. A significant negative correlation ($r = -0.64$) was found as shown in Figure 2. This relationship indicates that a high diastolic blood pressure is associated with the formation of smaller quantities of cortisol per gram of incubated slices.

In Figure 3 the formation of steroid in all positions has been related to diastolic blood pressure, Smithwick classification, albuminuria, and eye ground changes. It can be seen that a significant fall in Compound F ($\mu\text{g/gm}$) is associated with an increase in the severity of the hypertension as graded by the Smithwick classification. Decreases in the steroid formation of the before F and E positions, similar to that of cortisol, were also associated with an increased severity of hypertension. Significant increases of albuminuria and eye ground changes were also associated with decreased steroid formation as seen in Figure 3.

SUMMARY

1 Mean values of steroid formation ($\mu\text{g/gm}$) by adrenal slices of 16 hypertensive patients were larger in all 5 areas of the chromatograms studied than that of adrenals from 6 patients with carcinoma. Increased steroid formation was particularly pronounced on the B and S position.

2 Steroid formation at the B and S position in adrenal incubates of hypertensive patients, and at the E position of adrenal incubates of carcinoma patients, did not increase with the addition of ACTH as the steroid formation of the other positions did.

3 Cortisol formation by adrenal incubates of hypertensive patients fell into a low group (150–200 $\mu\text{g/gm}$) and a high group (300–400 $\mu\text{g/gm}$).

4 Decreased cortisol formation ($\mu\text{g/gm}$ of adrenal tissue) was found associated with an increase in diastolic blood pressure, increased severity of hypertension (according to the Smithwick classification), increasing albuminuria, and increased severity of eye ground changes.

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ADRENAL CORTICOID OUTPUT IN SURGICAL PATIENTS WITH OBSTRUCTIVE JAUNDICE*

JOHN H SCHNEEWIND AND JOHN B FULLER

We have been studying urinary adrenal cortical hormone output in surgical patients in an attempt to assay the role of these vital substances in recovery following operation. Surgical procedures activate the adrenal to an increased hormone output approximately 80 per cent of which in man is reported to be in the form of hydrocortisone—one of the 17 hydroxycorticoids. The main functions of the 17 hydroxycorticoids are related to carbohydrate and protein metabolism and anti-inflammatory and anti-pyretic effects. Regulation of electrolyte and fluid balance appears to be primarily affected by hormones without a hydroxy group in the 17 position such as aldosterone which are the minor fraction of adrenal output.

Recent reports have called attention to the importance of the liver in the metabolism of the 17 hydroxycorticoids. Peterson and associates¹ administered both hydrocortisone and radioactive hydrocortisone to normal and cirrhotic patients. Following infusions of 200 mg of hydrocortisone free alcohol over a 20 to 30 min period to 18 normal patients they found that 50 per cent of the hydrocortisone had left the plasma in approximately 114 minutes. When hydrocortisone was infused into 8 patients with cirrhosis it required from 160 to 800 min for half of the steroid to leave the plasma. Other closely related steroids (cortisone, tetrahydrocortisone etc) did not show this delayed removal from the plasma. These data were felt to imply that the enzyme system in the liver responsible for the metabolism of hydrocortisone was selectively impaired in liver disease. Brown and associates² gave intravenous hydrocortisone to 11 normal patients and to 12 with liver disease. Six hours after the infusion the plasma levels of the patients with liver disease was over three times as high as the normals. The rate of disappearance of hydrocortisone was inversely proportional to the degree of liver damage as measured by BSP retention. These authors also found that the patients with liver disease excreted about 30 per cent less 17 hydroxycorticoids in the urine.

*From the Department of Surgery, University of Illinois, Research and Educational Hospital, Chicago, Illinois. These studies were supported in part by a grant from the Upjohn Company, Kalamazoo, Michigan. We hereby express appreciation to Dr H C Batson for valuable assistance in analyzing our data statistically.

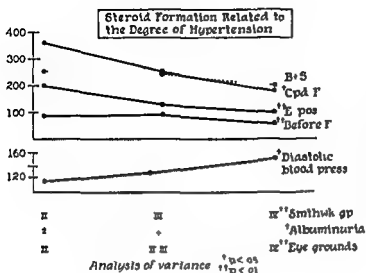


Fig. 3 Relationship of formation of Compound I and of the steroids in the Before I and II and S regions to increasing severity of hypertension in 12 patients. Statistical significance was computed by the analysis of variance.

Relationship Between Steroid Formation in Adrenal Incubates and Clinical Hypertension In an attempt to explain the separation of the cortisol formation into 2 definite groups the correlation of diastolic blood pressure to cortisone formation was studied. A significant negative correlation ($r = -0.61$) was found, as shown in Figure 2. This relationship indicates that a high diastolic blood pressure is associated with the formation of smaller quantities of cortisol per gram of incubated slices.

In Figure 3, the formation of steroid in all positions has been related to diastolic blood pressure, Smithwick classification, albuminuria and eye ground changes. It can be seen that a significant fall in Compound I ($\mu\text{g/gm}$) is associated with an increase in the severity of the hypertension as graded by the Smithwick classification. Decreases in the steroid formation of the before I and L positions similar to that of cortisol were also associated with an increased severity of hypertension. Significant increases of albuminuria and eye ground changes were also associated with decreased steroid formation as seen in Figure 3.

SUMMARY

1. Mean values of steroid formation ($\mu\text{g/gm}$) by adrenal slices of 16 hypertensive patients were larger in all 5 areas of the chromatograms studied than that of adrenals from 6 patients with carcinoma. Increased steroid formation was particularly pronounced on the B and S position.

2. Steroid formation at the B and S position in adrenal incubates of hypertensive patients and at the I position of adrenal incubates of carcinoma patients did not increase with the addition of ACTH as the steroid formation of the other positions did.

3. Cortisol formation by adrenal incubates of hypertensive patients fell into a low group (150–200 $\mu\text{g/gm}$) and a high group (300–400 $\mu\text{g/gm}$).

4. Decreased cortisol formation ($\mu\text{g/gm}$ of adrenal tissue) was found associated with an increase in diastolic blood pressure, increased severity of hypertension (according to the Smithwick classification), increased albuminuria and increased severity of eye ground changes.

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in 24 hr than normals despite normal Σ A M plasma levels. They concluded these data were evidence for decreased adrenocortical secretion in patients with liver disease in whom the rate of 17 hydroxycorticoid removal from plasma is impaired.

We wondered whether similar evidence of disturbed steroid metabolism would appear in surgical patients with obstructive jaundice. The data obtained from a study of 13 such patients as well as that on 11 normal patients (without evidence of obstructive jaundice or liver disease) form the basis of this report. Also included are results on 4 cirrhotic patients subjected to operation.

METHOD

Twenty four hour urines were collected from each patient for several days preoperatively during the day of operation and for the first 4 postoperative days.

The method used for the determination of urinary total 17 OH corticoids is that of Reddy, Jenkins and Thorn.³ Steroids are extracted with butanol after adjustment to pH 14 and quantitated by the Porter Silber reaction.

RESULTS

The data obtained indicate that the obstructive jaundice patients in this study excreted significantly smaller quantities of urinary 17 OH corticoids during the immediate postoperative period than did patients without obstructive jaundice or liver disease. A fairly typical example of our findings in a jaundiced patient is shown in Figure 1. At operation a cholecystectomy and common duct exploration were performed. Following a period of distention and ileus the patient suffered a wound dehiscence. After repair he gradually improved and was discharged on the sixteenth postoperative day.

OBSTRUCTIVE JAUNDICE ASSOCIATED WITH FLAT URINARY 17-OH CORTICOID CURVE

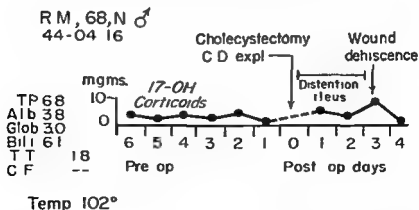


Fig 1 Urinary 17 OH corticoid excretion levels in a patient with gall stones and common duct obstruction. The daily output prior to operation was low and there was only a slight adrenal response following operation and after repair of a wound dehiscence. (Our figure for the first post op day in normal patients is 24 mg—see Fig 3.)

DELAYED STEROID EXCRETION AFTER CHOLECYSTOSTOMY

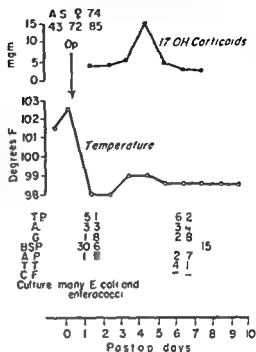


Fig 1 Urinary 17 OH Corticoid pattern in a critically ill patient following cholecystostomy for empyema of the gallbladder. Increase in adrenal output was minimal and did not appear until the fourth postoperative day. (The average output for normal patients on the first p.o. day was 24 mg—see Fig 3)

The 17 OH corticoid excretion pattern is unusually flat. There was no increase in steroid output preoperatively despite the cholecystitis nor was there any significant increase in corticoid output following operation.

A second example of corticoid patterns in jaundiced patients is shown in Figure 2. This 74 year old white woman was operated upon as an emergency because of an acute empyema of the gallbladder and a cholecystostomy was performed. The patient tolerated the operation rather poorly, having hypotension and irregularities of pulse and respiration. Following operation, however, she improved rapidly. The temperature returned to normal, her

AVERAGE URINARY 17-OH CORTICOID LEVELS AFTER OPERATION

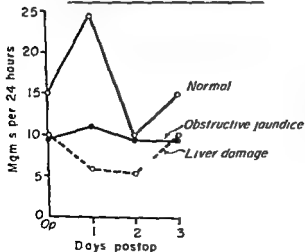


Fig. 3 Comparison of average urinary 17 OH corticoid output following operation. On the day of operation the normals averaged 15 mg and this increased to 24 mg on the first postoperative day. These outputs were 40 to 50 per cent greater than those for the obstructive jaundice group. Patients with liver damage had even lower outputs.

sensorium cleared and she was able to leave the hospital 11 days after operation with the cholecystostomy tube in place. The steroid output showed no response to the stress of the empyema of the gallbladder or operation until the fourth postoperative day when a moderate rise was noted. Following this the steroid levels promptly dropped to the previous levels.

The average urinary 17 OH corticoid output on the day of operation for the normal patients in this study was 15 mg. This rose to 24 mg on the first postoperative day, was 10 mg for the second day and 15 mg on the third day. In contrast the average output for jaundiced patients was 9 mg on the day of operation and 12, 9 and 9 mg on the first, second and third days respectively. These data are shown in graphic form in Figure 3 which also includes corticoid outputs of 1 cirrhosis patients following operation. The values for normal and jaundiced patients were subjected to an analysis of variance. The difference between total hormone output during the 4 days was significant at approximately the 5 per cent level ($P = 0.05$). Analysis was on only those patients with complete 4 day collections.

DISCUSSION

Normal Adrenal Response. Our findings on normal patients are consistent with currently held concepts of adrenal function. Operative trauma stimulates a rapid increase in adrenal output which is reflected in a rise in urinary excretion on the day of operation. Ordinarily the blood is cleared rather rapidly of excess amounts of steroid. Fifty per cent of an intravenous dose of radioactive hydrocortisone leaves the blood within $3\frac{1}{2}$ hr and 80 per cent appears in the urine during the first 24 hr.¹ The systemic effects of operation seem to alter the metabolism of the steroids so that the peak urinary excretion comes during the 24 hr following the day of operation.

Adrenal Response in Jaundiced Patients. Our results in these patients appear to corroborate the hypothesis that hepatic dysfunction has a direct relation to urinary steroid excretion. We have seen that even in normal patients operative stress retards urinary excretion due in part no doubt to alteration in hepatic function. The jaundiced patients in this series not only had appreciably lower outputs on the day of operation but failed to show a significant rise on the first postoperative day and in general had a flat type of excretion curve.

Adrenal Response in Cirrhotic Patients. This group also had low corticoid output following operation. The average output on the day of operation was 50 per cent less than normal patients. The levels declined on the subsequent 2 days and then returned to the operative day figure on the third postoperative day (Fig 3). This tendency for delayed steroid excretion in patients with liver disease is additional evidence of the importance of the liver in steroid metabolism.

"Baseline" Levels. By this term we mean the average daily urinary output prior to operation. The normal patients excreted 8.3 mg of 17 OH corticoids daily prior to operation which falls well within the normal range of one to 10 mg. The jaundiced patients had an average daily excretion of 4.7 mg. If this difference signifies depressed adrenal corticoid excretion in jaundiced patients then it may likewise imply an inability of the gland to respond to stress in normal fashion. In cases of borderline operability and during post operative complications this could be a critical deficiency.

SUMMARY

Urinary 17 OH corticoid levels in surgical patients have been measured before and after operation. Three groups of patients tested were patients with normal livers, those with obstructive jaundice, and a few with cirrhosis. We found a statistically significant difference in the hormone output after operation of these groups. Compared to the normals, the jaundiced patients excreted 10 per cent less on the day of operation and 50 per cent less on the first postoperative day. They also had smaller outputs on the second and third days after operation. The cirrhotic patients likewise had diminished urinary steroid levels. Prior to operation the normal patients excreted an average of 8.3 mg daily. The jaundiced patients excreted 17 mg daily, a difference of 43 per cent. These data support the concept of a direct relation between urinary 17 OH corticoid output and liver function. They also imply that hepatic dysfunction may result in adrenal depression which under conditions of severe stress might require adrenal replacement therapy for survival.

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Infections, Wounds and Burns

SOME STUDIES ON THE EFFICACY OF HETEROLOGOUS TETANUS ANTITOXIN*

A. BURGESS VIAL AND WALTER S. CALLAHAN

The common use of horse serum tetanus antitoxin and the frequent occurrence of individuals sensitized to horse serum lead to a high incidence of sensitivity reactions. These foreign protein reactions are difficult enough to contend with but there arises the question of whether the foreign serum antitoxin actually protects the sensitized individual as well as it does the nonsensitized. Certain work by other authors indicates that in diphtheria sensitized animals are not protected as well¹ and increasing doses of foreign tetanus antitoxin lead to increasingly rapid elimination of antitoxin.²

In order to test the question further antibodies against human serum were developed in rabbits. Tetanus antitoxin was developed in humans by repeated inoculations with tetanus toxoid. After mixing these serums and incubating them for an hour at 37°C it was found that the supernate had lost more than one half of its antibody titer as judged by the Ramon flocculation test.

*Table 1 Loss of Human Tetanus Antitoxin from
Supernate Flocculation Tests*

		RABBIT ANTI HUMAN SERUM	NORMAL RABBIT SERUM	155
Human	Test 1	56	22.5	22.5
Tetanus	Test 2	28	22.5	22.5
Antitoxin	Test 3	56	11.2	22.5

It was demonstrated in another way too that mixing human tetanus antitoxin and rabbit antibodies against human serum removed tetanus antitoxin from the supernate. The supernate was mixed with toxin and injected into mice and it was found that the supernate had lost most of its ability to neutralize the toxin.

The human tetanus antitoxin must then have been precipitated in large part by the anti human serum. It surprised the authors to find that the precipitated material still had some ability to protect mice against tetanus toxin although there was a great net loss of effective antibodies from supernate and precipitate. That the precipitated antitoxin still had any antitoxin activity might be explained by assuming that part of the antitoxin was carried down as part of the large antigen antibody aggregate without the antitoxin being intimately involved in the reaction or the antitoxin mole

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cule may have different combining sites for the toxin and for the anti human serum

*Table 2 Loss of Human Tetanus Antitoxin
Neutralization Tests*

	RABBIT ANTI HUMAN SERUM		P.S.
	SUPERNATE	PRECIPITATE	
Human Tetanus Antitoxin	19/25	20/25	0/5

Further tests were made mixing the human antitoxin and antibodies against the human serum. After the mixture had been incubated at 37°C for an hour with constant shaking the whole material (i.e. bound and unbound antitoxin) was injected intramuscularly into mice; the mice were then challenged with a given dose of toxin. In one test for example $\frac{1}{10}$ of the mice so treated died in 96 hr. while only $\frac{2}{10}$ of each of the control groups died.

*Table 3 Loss of Human Tetanus Antitoxin
Protection Tests
(Mice Dead in 96 Hours)*

	RABBIT	NORMAL	P.S.
	ANTI HUMAN SERUM	RABBIT SERUM	
Human Tetanus Antitoxin	7/10	2/10	2/10

This suggested that some of the antitoxin molecules were not just temporarily bound by the antibodies against the antitoxic serum, but rather that the effect of the antitoxin was definitely reduced.

When this last experiment was repeated, but with intravenous administration of the mixture of human tetanus antitoxin and anti human serum, the protective power of the antitoxin was again demonstrated to be reduced.

It seemed pertinent to try to imitate the problem of the sensitized human by using animals administering heterologous antitoxin and then ascertaining the antitoxin levels periodically. Guinea pigs were immunized with rabbit serum and then rabbit tetanus antitoxin was given the guinea pigs. Controls consisted of unsensitized guinea pigs administered the same amounts of rabbit tetanus antitoxin. The guinea pigs were then periodically bled and their antitoxin titers assayed. Although the guinea pigs previously immunized with rabbit serum had initial antitoxin titers as high as the controls, the titers fell more rapidly than did those of the controls.

Judging from this test the guinea pigs previously immunized with rabbit serum were less well protected by the rabbit antitoxin.

CONCLUSIONS AND SUMMARY

Heterologous tetanus antitoxin is less effective in animals immunized against the heterologous serum. This was demonstrated by the fact that after an animal is immunized with heterologous serum his blood precipitates out

FALL OF PASSIVE ANTITOXIN TITERS IN GUINEA PIGS

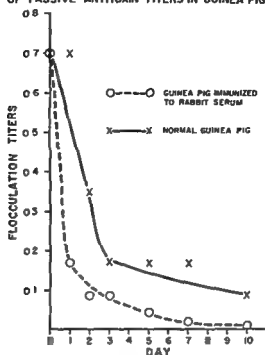


Fig 1

A large part of the heterologous antitoxin. A part of this precipitated antitoxin can be recovered as effective antitoxin from the precipitate. Administration of heterologous antitoxin to an animal immunized against the heterologous serum is followed by a more rapid loss of the antitoxin from the animal's circulation than from the serum of the control animal which has had no previous heterologous serum.

This leads to the conclusion that human tetanus antitoxin would likely protect certain humans better than would animal antitoxin of similar titer. It should be pointed out, however, that production of human tetanus antitoxin of high titer has not yet been accomplished.

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THE CONTROL OF POSTOPERATIVE WOUND INFECTIONS DUE TO ANTIBIOTIC RESISTANT STAPHYLOCOCCUS AUREUS*

CHESTER W. HOWE

Recent publications^{1, 2} and discussions with various surgeons indicate that in some surgical units epidemics of postoperative sepsis owing to *Micrococcus Pyogenes* (var. *aureus*) have reached serious proportions. Attention was called in a previous paper³ to the increasing number of antibiotic resistant strains of this organism being recovered from hospital patients and personnel throughout the world since the introduction of penicillin. Numerous surveys and bacteriophage typing studies indicate that the widespread use of penicillin has reduced the number of sensitive strains and allowed resistant staphylococci to flourish and become predominant. These resistant strains are then disseminated from patients to carriers and thence to other patients. This newly introduced phenomenon—namely the dissemination of drug resistant bacteria—has thus far been limited to hospitals and there is no evidence that it has affected the general population. To maintain a high incidence of resistant strains among carriers a large number of patients must be receiving the drug. This condition is fulfilled on a surgical service where penicillin is used heavily and constitutes one of the special situations where an increased number of staphylococcal outbreaks are being observed.

The purpose of this paper is to describe our experience with postoperative staphylococcal sepsis since 1949 and the measures we have taken to control it.

METHOD

Accurate statistics are available on the incidence of postoperative wound sepsis following clean operations on the house and private surgical services of the Massachusetts Memorial Hospitals since 1949. All operations are classified as to their expectancy as regards sepsis at the time of operation.⁴ Procedures where no contamination or infection is expected or justifiable as for example herniorrhaphy or sympathectomy and operations in which there is actual or potential contamination but in which wounds are normally expected to heal by primary intention such as cholecystectomy or intestinal resection are considered as clean. Infections are classified as major if they cause systemic manifestation, progressive invasion or destruction of tissue, prolonged hospitalization or spontaneous or induced drainage of pus requiring therapy. Minor infections are those showing only localized inflammatory reaction in skin margins, stitch abscesses or a trivial localized area of sepsis. The diagnosis of infection is made on a clinical basis in the closed postoperative wound. Infection is not diagnosed on the basis of a positive culture alone.

One large ward and the operating room floor were chosen as a sample cross section on which to perform a check of the hospital carrier rate of *Staphylococcus aureus*. On all persons appearing in these areas during a 24 hr period cultures of the nasopharynx and skin of the right hand were taken.

*From the Departments of Surgery, Boston University School of Medicine and the Massachusetts Memorial Hospitals. Supported by a grant from the Trustees under the wills of Charles A. King and Marjorie King.

Floras were tested for sensitivity by placing a 1 unit penicillin disc* on blood agar plates freshly inoculated from the culture swabs. Zones of inhibition 11 mm or less after 24 hr incubation at 37°C were considered resistant. All others were considered sensitive. If a large inoculum (solid sheet of growth) was not present the test was repeated with a heavy growth after subculture.

All results were examined by Mr. John Alman, director of Statistical Research Services at Boston University. Chi-square analysis or the T test using the angular transformation were applied as indicated.

RESULTS

Infection and Carrier Rates prior to 1953 Table 1 shows that the rates for major and minor infections combined was 1.09 per cent of the clean operations in 1949 but that there was a gradual stepwise statistically significant increase to 3.98 per cent in 1953. This occurred despite the widespread prophylactic use of antibiotics. As in most hospitals the infection rate for house service cases was considerably higher than for private ones. Analysis of the nutritional status, age, weight, blood chemistry and hematological findings and type of surgery showed no significant difference in the clinical status between 1949 and 1953.

Almost all the increase in infections over this 5 yr period was owing to major sepsis. Table 2 shows the rates for major infections, the ratio of infections caused by *Staphylococcus aureus* to those caused by other organisms and the percentage of infections caused by this organism in the wounds that were cultured. The rate increased from 0.58 per cent in 1949 when 57.1 per cent of the infections were caused by *Staphylococcus aureus* to 2.85 per cent

Table 1 Rates of Major and Minor Infections After Clean Surgery

		HOUSE SERVICE	PRIVATE SERVICE	BOTH SERVICES
1949	No Operations	401	796	1197
	% Infections	1.99%	0.63%	1.09%
1950	No Operations	379	750	1129
	% Infections	3.16%	1.06%	1.77%
1951	No Operations	366	843	1209
	% Infections	3.82%	1.18%	1.98%
1952	No Operations	388	803	1193
	% Infections	5.15%	1.24%	2.51%
1953	No Operations	429	800	1229
	% Infections	7.22%	2.25%	3.98%
<i>New Program Started</i>				
1954	No Operations	326	806	1132
	% Infections	5.21%	1.61%	2.65%
1955	No Operations	321	845	1166
	% Infections	5.29%	0.94%	2.14%

*Dia discs: Commercial Solvents Corporation, New York City.

Table 2 Major Infection Rate and Incidence of Staph Sepsis for Clean Surgery

YEAR	NO OF CLEAN OPERATIONS	NUMBER	MAJOR WOUND INFECTIONS			OWING TO	
			INFECTION RATE (PERCENT)			STAPH AUREUS	
			HOUSE SERVICE	PRIVATE SERVICE	AVERAGES	RATIO	PERCENT
1949	1197	7	1.21	0.25	0.58	1.7	57.1
1950	1129	12	2.10	0.53	1.06	7.12	58.3
1951	1209	17	3.00	0.71	1.11	7.17	41.2
1952	1193	20	3.09	0.99	1.68	10.17	58.8
1953	1229	32	4.66	1.87	2.82	25.31	80.6
New Program Started							
1954	1152	18	3.37	0.86	1.59	15.17	88.2
1955	1165	18	3.42	0.82	1.54	9.16	56.2

Table 3 Staphylococcal Nose and Skin Carrier Surveys

DATE	NO OF CULTURES TAKEN			STAPH CARRIERS		PENICILLIN RESISTANT STRAINS
	PATIENTS	PERSONNEL	TOTAL	NUMBER	PERCENT	
Aug 1953	37	89	125	124	99.2%	78.5%
Aug 1955	12	156	178	134	75.3%	82.7%

in 1953 when 80.6 per cent of them were owing to this organism. Table 3 shows that 99.2 per cent of the sample of patients and personnel surveyed in August 1953 were *Staphylococcus aureus* carriers and that 78.5 of these were carrying penicillin resistant strains.

Corrective Measures During the latter part of 1953 and early 1954 various measures to prevent cross contamination and the dissemination of antibiotic resistant bacteria were inaugurated as follows: (1) The use of routine antibiotic prophylaxis was abandoned on the house service and largely curtailed on the private service. Antibiotics were reserved for the treatment of established infections. (2) Since the nasopharynx is the natural reservoir for staphylococci, the use of two face masks (one over the other) during operations and the changing of these masks by the circulating nurse every 1½ hr was urged. (3) A full 10 min scrub with a G-11 soap at least for the first case was urged. (4) The established principles of meticulous skin preparation, gentle surgery, dry wounds, and careful closure were emphasized. (5) The use of face masks for doctors, nurses, and patients and of gloves during changing of dressings on the wards to help prevent the personnel from becoming carriers was instituted. (6) Special septic sets containing instruments in an aluminum pan covered by a sterile double ply cloth wrapper were provided. Soiled dressings were deposited in a large waxed paper bag placed in a closed container and incinerated daily. At the conclusion of the dressing, instruments, gloves, and other soiled material were placed in the pan which was wrapped and autoclaved before cleaning and definitive steril-

ization (7) Strict aseptic technique as regards the dressing truck (8) Local isolation of infected wounds by impermeable occlusive dressings (isolation of patients proved impracticable) (9) Prompt control of infected wounds by surgical drainage and debridement under antibiotic protection with early secondary closure where feasible.

These measures were introduced over a period of several months and participation by the staff was generally good with some lapses by a few individuals.

We are also aware of the possible role of fecal carriers, blankets, laundry and various housekeeping factors as sources of contamination. No studies or measures directed toward these sources have been instituted as yet.

Infection and Carrier Rates after 1953 Table 1 shows that following the introduction of this program the overall infection rate dropped from 3.99 per cent in 1953 to 2.65 per cent in 1954 and to 2.11 per cent in 1955—a reduction by almost half which is highly significant statistically. Table 2 shows that in 1955 the major infection rate fell to 1.51 per cent and in that year the proportion of infection owing to *Staphylococcus aureus* was down to 56.2 per cent.

In August 1955 a survey using the patients and personnel in the same areas previously described was repeated using identical technique. Table 3 compares these surveys and shows that the carrier rate was reduced from 99.2 to 75.3 per cent in the 2 year period and that the incidence of penicillin resistant strains in carriers fell from 78.5 to 62.7 per cent—both significant reductions.

DISCUSSION

The staphylococcus has always been a common cause of wound sepsis and was found in 16 per cent of Melencz's clean wound infections over an 8 yr period in the pre-antibiotic era. Our rate of 80.6 per cent in 1953 appears therefore to be quite high and its reduction to 56.2 per cent in 1955 approaches pre-antibiotic era levels. Various studies¹ on the staphylococcal nasopharyngeal carrier rates of hospital patients and personnel before 1941 show ranges from 22 to 45 per cent so it would appear that our 1955 levels although improved are still abnormally elevated. Many of the constituents of the normal respiratory flora are non-pathogenic for wounds, low in virulence or penicillin sensitive. When there is a high hospital carrier rate of *Staphylococcus aureus* there is a greater chance for wound infection to develop because when contamination occurs there is an increased likelihood that it will be with this virulent organism rather than some of the non-pathogens it has replaced.

Examination of these data suggest that the increase in sepsis up to 1953 was related to an abnormally high carrier rate of penicillin resistant *Staphylococcus aureus* as a result of the widespread and prolonged use of penicillin and it is postulated that routine and extensive use of prophylactic antibiotics might therefore have an unfavorable effect on the postoperative infection rate.

Improvement has followed the use of relatively simple and well known measures designed to prevent wound contamination and cross infection. It appears likely that this improvement is related in some degree to the preventive measures outlined.

The higher rate of infection in house service patients is our main problem and is currently being studied. The inexperience of the resident staff is probably partly to blame but there are numerous other factors which may be contributory. The house officer spends most of his time in the hospital environment, does most of the dressings and has greater opportunity than the visiting surgeon to come in contact with resistant organisms and become a heavier carrier. Private operations are likely to be shorter and the patients are apt to be in single rooms and hence less susceptible to cross contamination than those on the wards. Host factors probably play an important role. Since the development of medical insurance and Veterans Administration plans the majority of house patients are in the older age group. Young good risk patients with uncomplicated surgical diseases are seldom seen on our wards at present.

It is known that staphylococcal resistance will develop with other antibiotics than penicillin and that the development of resistance to an antibiotic is roughly in proportion to its use in a given area. The resistance pattern of a bacterial species therefore may vary from time to time depending upon the antibiotic being used most extensively in a hospital.

SUMMARY AND CONCLUSIONS

1 Data on the clean wound infection rate at Massachusetts Memorial Hospitals for the period 1919-1955 are presented. A high hospital carrier rate of penicillin resistant *Staphylococcus aureus* is a result of widespread use of this antibiotic is regarded as an important contributory factor toward the gradually increasing infection rate during 5 yr prior to 1951.

2 During the last 2 years the carrier rate of this organism and the penicillin resistant strains in carriers have been significantly lowered and the postoperative wound infection rate has been reduced by almost half following institution of a relatively simple program for the prevention of cross infection, the judicious use of antibiotics and meticulous wound management.

3 The extensive prophylactic use of antibiotics in a hospital by virtue of the emergence and dissemination of resistant strains may intensify the problem of staphylococcal sepsis to such an extent as to contribute significantly toward an increase in the overall infection rate.

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EVALUATION OF PERITONEAL LAVAGE IN THE TREATMENT OF SEVERE DIFFUSE PERITONITIS

G RAYMOND BROWN JR AND W EMORY BURNETT

The purpose of this experiment was to produce uniformly severe, diffuse peritonitis in a laboratory animal, which compared with clinical human peritonitis and to treat it by the various methods by which peritonitis is handled in the operating room

Guinea pigs were chosen as the subject of this experiment as they were readily available small relatively inexpensive, and less resistant to infection than rats or mice used by others They were obtained from one source and were approximately 6 wks of age Their average weight was 290.5 gm

Anesthesia consisted of veterinary pentobarbital sodium intra peritoneally Dosage was calculated at 30 mg/kg with the majority receiving 6.5 mg Duration of anesthesia was from 2 to 4 hr When an animal was reoperated upon the same day a second dose of 6.5 mg 1 hr after the first showed few complications The intra peritoneal route may be questioned but in none of the cases was there any evidence of contamination from the needle puncture Failure of anesthesia in several cases was thought to be due to intraluminal injection of the pentobarbital

A method of causing a uniform lethal peritonitis was sought one in which the animals would survive long enough that remedial therapy could be instituted Initially a 1 cm oval portion of the large cecum was excised but death occurred in 3 to 4 hr Then ellipses of various lengths were removed from the colon The larger the ellipse the sooner death occurred It was decided that an incision in the colon might be better but these sealed off rapidly with more than 50 per cent of the animals surviving

Finally it was found that an ellipse 3 mm in length in the colon caused death in 17 of 20 guinea pigs and that 16 of these died in less than 24 hr All subsequent groups were therefore given peritonitis by this method

All guinea pigs were observed post operatively for a period of at least one week No special care was given except as noted below Death occurring post operatively within 2 hr automatically eliminated an animal from this series

Peritoneal cultures were taken in all groups Peritonitis was due to *E. Coli*, *B. Subtilis* and *Streptococcus fecalis* (enterococcus) and sensitivity tests showed that these bacteria were susceptible to penicillin and streptomycin in combination Post mortem examinations were done routinely and bacteriological studies were taken at that time An estimate of the amount of soiling present was made at the second operation

To show the results following variations in therapy 6 groups of 20 guinea pigs each were given peritonitis by the above method All groups were reoperated upon 4 hr after the onset of peritonitis and the ellipse in the colon was repaired using #60 chromic catgut continuously and #50 silk interrupted sutures Gross local contamination was removed and after specific therapy was carried out the abdominal wound was closed

Group 1 was reoperated upon as described above The ellipse in the colon was repaired Local contamination was removed and the abdominal wall was closed No further therapy was given and 7 of the 20 animals died All peritoneal cultures were strongly positive

Group 2 was reoperated as above the bowel was repaired similarly local contamination was removed and prior to closing the peritoneum 5000 units of crystalline penicillin and 25 mg of streptomycin in 5 cc. of saline were instilled. Again 7 of the 20 animals died. Peritonitis was less severe by culture than in Group 1.

Group 3 was treated as before. However the only therapy used was procaine penicillin C 30 000 units and streptomycin 50 mg intramuscularly for 2 days. Only 5 of the 20 animals died and cultures taken at post mortem were sterile in 3 and only a few bacteria were seen on the other 2.

Group 4 was prepared as previously with the exception that the peritoneal cavity was lavaged copiously with warm normal saline until gross contamination was removed. Five of the 20 animals died and cultures showed marked peritonitis at post mortem.

Group 5 was reoperated upon as before and the peritoneal cavity was lavaged with saline until gross contamination was removed and then re-lavaged with saline containing 100 000 units of crystalline penicillin and 5 gm of streptomycin per 500 cc. Six of the 20 animals died but of these 2 died on the third day of clinical and bacteriologically proven pneumonia.

Group 6 was prepared similarly and 4 hr after the onset of the peritonitis they were reoperated the ellipse in the colon was repaired and the peritoneal cavity was lavaged with copious amounts of warm saline containing 100 000 units of crystalline penicillin and 5 gm of streptomycin per 500

Table 1 Summary of Results

GROUP	TOTAL	DEAD	ALIVE	AMOUNT OF PERITONITIS BY CULTURE POST MORTEM	ESTIMATED AMOUNT OF SOILING
INITIAL (peritonitis only)	20	17	3	SEVERE	
1 CLOSURE ONLY	20	7	13	SEVERE	ABOVE AVERAGE
2 CLOSURE AND INSTILLATION P&S	20	7	13	MODERATE	ABOVE AVERAGE
3 CLOSURE AND P&S IM	20	5	15	MILD	AVERAGE
4 CLOSURE AND LAVAGE WITH NaCl	20	5	15	SEVERE	AVERAGE
5 CLOSURE AND LAVAGE WITH NaCl AND P&S	20	6*	14	MILD	AVERAGE
6 CLOSURE AND LAVAGE WITH NaCl AND P&S IM	20		20	NONE	AVERAGE

(* 2 died of pneumonia)

cc The abdominal cavity was then closed and intramuscular procaine penicillin 30 000 units and streptomycin 50 mg were given daily for 2 days. None of the 20 animals died.

SUMMARY

Severe diffuse peritonitis caused by removing an ellipse of colonic wall was found to be fatal in 17 of 20 guinea pigs.

Six other groups of 20 guinea pigs each were prepared in this manner and were reoperated upon 1 hr later. Each group was treated in a different manner.

It was found that: 1. Removal of the cause of the peritonitis alone reduces the fatality markedly. 2. The amount of peritoneal soiling present at the time of operation is directly proportional to the mortality rate. 3. Any further therapy besides removal of the cause of the peritonitis decreased the mortality. 4. Local cleansing of the area of peritoneum involved and instillation of penicillin and streptomycin after removal of the cause of the peritonitis does not produce as good results as peritoneal lavage with saline without antibiotics and removal of the cause. 5. The best results in the experiment were found in the group of animals in which the peritonitis was treated by peritoneal lavage with penicillin and streptomycin in saline and with that combination of antibiotics intramuscularly.

THE HANDLING OF ABDOMINAL WOUNDS UNDER MASS CASUALTY CONDITIONS*

An Experimental Study

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In the event of a thermonuclear disaster, Civil Defense and military personnel estimate that there will be no evacuation for 8 hours and no organized medical care for at least 12 hours following detonation. In view of the overwhelming number of casualties, medical personnel will be vastly inadequate and a policy has been formulated whereby several types of injuries will be set aside at the sorting station. These will receive no therapy until those with greater chances of survival have received treatment. These authorities justifiably feel that all surgery will necessarily be performed by inexperienced personnel under the supervision of a surgeon and that no more than 30 minutes can be allotted to each operation. It is thought that this is insufficient time to perform life saving measures in the case of abdominal wounds.¹ Therefore

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these will be placed in the group to receive delayed therapy. No factual basis for this policy could be found and it was apparently formulated on the basis of Civil War experience.¹ This experiment is designed to assess the problem under simulated disaster conditions.

METHOD

One hundred and eighty mongrel dogs were lightly anesthetized with 0.5 per cent intravenous thiopental sodium. One hundred and forty-four of these animals were shot with a .22 caliber target pistol using .22 caliber short ammunition with an estimated muzzle velocity of 1000 ft/sec. The animals were shot in three separate groups. They were placed on their sides over a sandbox and shot in the flank midway between the spinous processes and the midline of the abdomen. Groups 1 and 3 received wounds of entrance at the level of the 11th and Group 2 at the level of the 5th lumbar vertebra. Wounds of exit were directly opposite. In Group 1 (60 animals) there were 12 anesthetic controls that were not shot but were anesthetized each time the animals were operated upon. Groups 2 and 3 were composed of 60 animals each with 12 animals in each group being anesthetized only at the time of shooting.

The animals were separated at the time of shooting into a group to be treated and a control group that was to receive no therapy. The 12 animals in each control group were placed in cages and observed. Animals in the group to be treated were placed in a large room with no food or water for a period of 12 hours. At the end of 12 hours the surviving animals were divided into 2 equal groups. 1 group being operated upon immediately, the survivors of the other group being operated upon at 36 and 60 hours. None of the animals were given any supportive therapy or antibiotic.

Operation. Animals were anesthetized with a continuous intravenous drip of 0.5 per cent thiopental sodium and the shaved abdomen was prepared with iodine and alcohol. The surgical teams were composed of 2 third year medical students with no previous surgical experience. They donned caps and masks and scrubbed using a one brush technique. Sterile gloves but no gowns were worn. Each team had available 4 sterile towels for draping, 1 towel clip, 6 hemostats, 1 knife, 2 thumb forceps, 1 pair of scissors, 1 needle holder, 1 one half curved Murphy and 1 Keith needle, 1 small retractor, 40 twisted silk and 20 4x4 gauze sponges. One qualified surgeon supervised and a circulating diener was available for each 5 teams. Operating time was limited to 30 minutes.

The abdomen was opened through a long vertical incision. A quick evaluation of the situation was made and the problem discussed with the supervising surgeon. Perforated bowel was closed if this could be done with 1 to 2 sutures. Large segments of necrotic bowel were excised using mass ligation techniques and the ends exteriorized. Where large perforations were present that segment of bowel was exteriorized. Damaged spleens were removed again using mass ligation technique. Bladder perforations were closed quickly if they were small or if large the bladder wall was sutured to the skin. The abdomen was closed using doubled 40 twisted silk as through and through sutures. All exteriorizations were made through stab wounds.

All animals except 3 in the non-operated group were autopsied. These unfortunately were lost.

Table 1 Injuries by Site

SITE OF INJURY	GROUP 1		GROUP 2		GROUP 3	
	CONTROL	OPERATION	CONTROL	OPERATION	CONTROL	OPERATION
Small bowel	6	21	5	14	1	14
Small bowel spleen	5	8	1	2	7	8
Small and large bowel		1		3	1	3
Small bowel bladder				3		1
Small bowel spleen bladder				1		
Small bowel mesentery						1
Small bowel uterus		1				2
Uterus						1
Uterus bladder						1
Bladder mesentery				1		
Large bowel				1		
Bladder	1	1	3	4		4
Broad ligament				1		
Omentum		1		1		
Abdomen				5		1
Not specified			3			
TOTAL	12	36	12	36	12	36

RESULTS

Sites of Injury These are shown in Table 1. The animals that expired prior to 12 hours had predominantly bowel injuries and injuries to the spleen while those that survived more than 12 hours had a higher incidence of injuries to other organs such as bladder, broad ligament, uterus, omentum and mesentery.

Survival In the control group 26 dogs were living at the end of 12 hours with the ultimate recovery of 3—a survival rate of 12 per cent. These 3 ultimate survivors had unspecified injuries. In the group for operation 50 dogs survived 12 hours with the ultimate recovery of 12—a survival rate of 24 per cent. In these survivors there were 5 bladder injuries, 5 abdominal wall injuries, 1 broad ligament injury and 1 omental injury. These results are shown in Table 2. Five out of 30 animals operated upon at 12 hours, 5 out of 9 at 36 hours and 2 out of 3 at 60 hours survived as shown in Table 3.

Mortality The overall mortality in the first 12 hours in all animals that were shot was 47 per cent. In the operative group no animal with a bowel perforation or uterine injury survived. It is also interesting to note that no animal with perforated bowel survived as long as 36 hours without operation. There were 2 deaths in the anesthesia controls which were used in Group 1 and there were 2 deaths in the remaining 21 animals giving an anesthetic mortality of 12.5 per cent.

DISCUSSION

Howard and DeBakey² reported a case fatality of 12.6 per cent in abdominal wounds receiving early treatment in Korea with a calculated increase in fatality of 0.5 per cent per hour delay of treatment. Average operating time

Table 2 Outcome of Injured Dogs that Survived 12 Hr, by Site of Injury and Handling

SITE OF INJURY	OPERATION GROUP I		OPERATION GROUP II AFTER OPERATION IF SURVIVING		CONTROL GROUP I NO OPERATION	
	12 HR SURVIVAL	ULTIMATE SURVIVAL	12 HR SURVIVAL	ULTIMATE SURVIVAL	12 HR SURVIVAL	ULTIMATE SURVIVAL
Bowel injuries	19		10		20	
Uterus injuries	1		3			
Bladder alone	6	3	2	2	3	
Other injuries	4	2	5	5	3	3
TOTAL	30	5	20	7	26	3

Table 3 Survival Figures

	CONTROL GROUP I	OPERATION GROUP			12 HR SURVIVALS	
	12 HR SURVIVALS	12 HR	36 HR	60 HR	NONE	TOTAL
Number of dogs	26	30	9	5	8	50
Number recovering	3	5	5	2		12
% recovering	12%	17%	56%	67%		24%

per abdominal case was 24 hours. The conditions for evacuation and care of patients plus the inevitable presence of complicating associated injuries in the event of a thermonuclear disaster will without doubt increase the mortality rates which they cited. However under survival conditions the operating times can be greatly lessened.

The high first 12 hour mortality rate in this experiment is undoubtedly due to untreated hemorrhage and shock coupled with the fact that the very vascular spleen is disproportionately large and mobile in the dog. To simulate the lack of competent anesthetists intravenous sodium thiopental drip anesthesia was selected and its control was by the supervising surgeons or circulating dieners. This must have increased the mortality in the group receiving surgery. However it must be noted that these were relatively clean wounds as compared to those one might see from flying debris. The injured who survive immediate radiation flash or blast effects will in all likelihood receive wounds from secondary missiles at a velocity of approximately 800 ft/sec accompanied by vast quantities of dirt.¹

Although in this experiment ultimate survival 12 hours after receiving an abdominal wound is increased from 12 to 24 per cent by surgical intervention the justification for this procedure is conjectural. Only surgically trained and aware personnel in the sorting stations can make the final decision under disaster conditions.

CONCLUSIONS

1 Under the conditions of this experiment inexperienced personnel under supervision were able to perform adequate abdominal exploration and certain specified surgical procedures in 30 minutes.

2 Such surgery does not improve the survival rate in bowel injuries but seems to improve the survival rate in other abdominal injuries

3 The high mortality in the first 12 hours coupled with the relatively slight improvement following operation confirms the justification of a policy of delayed evacuation of the abdominally wounded

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THE USE OF NYLON TAFFETA IN THE REPAIR OF ABDOMINAL WALL DEFECTS*

ERIC M. NANSON AND GAETANO T. FIORICA

The majority of abdominal wall defects can be repaired with the use of adjacent tissue but when large defects exist it may be impossible to approximate the edges of normal tissue without marked tension. In these cases it may be necessary to use implants of inert materials. In the past autogenous material has been used but its limitations has led to increasing interest in the use of non living material many of these materials have now been used for a considerable length of time.

The inert materials used fall into two groups metallic and plastic. Metallic meshes have been most widely used but despite improvement in making these metals more biologically and chemically inert they are still liable to fracture due to their constant movement in living tissue. The more pliable plastic materials appear to have overcome this inherent weakness of the metals.

Of the various available plastics nylon has been the most widely used during the last few years. Nylon is a material obtained by the synthesis of carbonium. It has an homogeneous aspect and consistency. Its specific weight is 1.14 and it liquifies at 250°C. Its advantages are that it is biologically and chemically inert. Being unaffected by minor changes in pH it can be sterilized easily by boiling or by autoclaving. It appears to be durable and provides a framework within the tissues. It has a high malleability and does not appear to fracture with constant flexion. It is smooth, radio translucent, inexpensive and readily available.

The use of nylon mesh in the repair of herniae was first reported by Acquaviva and Bourret in 1944.^{1, 2} Together with Cuture they used a fine mesh monofilament nylon sheet. The individual filaments of the mesh were very thin (0.2 mm. to 0.5 mm.) but were reported to be strong.

Burmam³ in 1948 published the results of 6 years work using nylon mesh in the treatment of very large umbilical herniae.

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Corti⁶ in 1919 reported cases of large abdominal wall herniae in females which he treated with nylon mesh. One of his patients later had Cesarean section and the operative incisions were made through the nylon inlay. He reported that the incisions were not difficult to close and that the abdominal wall could be sewn in the usual manner. His results were satisfactory. Similarly in 1951 Aquino⁷ reported his experiences using nylon in repairing 30 large hernia defects. He commented that the repair was simple, that the nylon appeared inert, and that the procedure seemed valuable in places which would have been treated with difficulty by other methods.

Carand, Vittori and Toucher⁸ (1950) reported 6 cases. They stressed the importance of careful hemostasis and asepsis when using nylon inlays as 2 of their 6 cases became infected. Despite the infection the hernia inlays were intact. Stock in 1951 reported excellent results using wide mesh nylon in 10 inguinal hernias and 2 abdominal wall defects.

In the experimental field Testa⁹ in 1951 used nylon to repair artificially created abdominal defects in animals. The created defects consisted of all muscular and fascial layers of the abdominal wall. He reported that nylon appeared to produce a minimal tissue reaction and that it appeared to be well tolerated by the tissues. His experiments were conducted on rabbits.

Moore and Siders⁷ in 1955 published the results of using nylon to repair similar defects in the anterior abdominal walls of dogs. In half the animals they used 3 layers of impervious nylon polyethylene sheeting as a graft. In some of the dogs they replaced the peritoneum with this inlay. The results of this maneuver were not satisfactory because of the high incidence of suture line dehiscence, fluid accumulation, and marked tissue reaction. However, they then went on to use fine mesh nylon in replacing various layers of the anterior abdominal wall including the peritoneum. They found the results much improved in that the nylon mesh was better tolerated than the impervious sheeting. They noted that the interstices of the grafts were invaded by fibroblasts thus incorporating the graft in the surrounding tissues.

Reviewing these reports it appears that nylon mesh may be the material of choice in surgical repair of large abdominal wall defects. The mesh can either be used to reinforce normal but weak tissue planes when the edges of the wound can be opposed or, if apposition is impossible, it can be used unsupported to substitute for the muscular and fascial layers and in some instances may be the only tissue between skin and peritoneum. Although the nylon appears to be inert and is well tolerated by the tissues, it does cause a mild inflammatory reaction in the first few months. This goes on to a fibroblastic reaction which permeates the mesh. The resulting scar tissue strongly reinforces the nylon inlay.

METHOD

In order to assess the validity of the above claims for the use of nylon mesh in hernial defects, the following experiments were carried out in the Experimental Laboratory of the University Hospital.

Abdominal wall defects involving various layers of the abdominal wall approximately 8 cm. by 4 cm. were created in 32 healthy animals—16 dogs and 16 rabbits. These defects were then repaired as shown in Table I.

All animals were operated upon under intravenous nembutal anesthesia at a dose of 30 mgm./kg. of body weight. In all cases the abdomen was shaved

Table 1 Scheme of Nylon Taffeta Repair of Abdominal Wall Defects with Results

		GRAFT REMOVED		RESULTS
		AT 3 MOS	AT 6 MOS	
Ant Rectus Sheath only	Dogs	5	3	Satisfactory
Rectus Muscle with Ant & Lost Rectus Sheath	Rabbits	5	3	Satisfactory
Peritoneum only	Dogs	5	3	Satisfactory
Peritoneum only	Rabbits	5	3	Satisfactory

and disinfected with tincture of iodine 2½ per cent prior to the operation. Postoperatively all animals were given antibiotics for 7 days by intramuscular injection. In the majority of cases acromycin was used but in a few animals a penicillin streptomycin mixture was used.

Anterior Rectus Sheath Only In 8 healthy dogs a rectangular piece of the anterior rectus sheath was excised. The defect was then repaired with a rectangle of nylon taffeta a little smaller than the defect so that it was under tension. The nylon graft was sutured to the fascial sheath using interrupted 3/0 silk. The dogs made an uneventful postoperative recovery. Moderate inflammatory reaction with edema was noted in all cases in the area of the operation for about 10 days. This quickly settled but in 3 of the 8 dogs some thickening appeared to persist in this area. In the other dogs the abdominal walls appeared quite normal. After 3 months 5 of the dogs were sacrificed. These included the 3 dogs which showed some residual inflammatory reaction. The grafts were dissected out and subjected to histologic examination. Macroscopically the graft was found to be incorporated in a sheet of fibrous tissue which was well united with the surrounding anterior rectus sheath. In all 3 of the dogs that had shown clinically some persistent inflammatory reaction purulent exudate was found around the nylon but this had not interfered with the production of a strong sheet of fibrous tissue. On microscopy the nylon mesh was surrounded by a narrow sheet of dense fibrous tissue which was infiltrated with a small number of lymphocytes and histiocytes (Fig. 1). This infiltration was most marked immediately adjacent

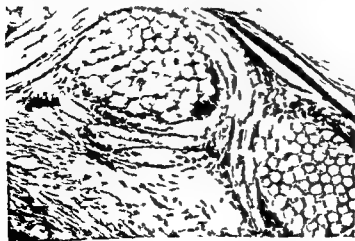


Fig. 1 Microscopic appearance of nylon taffeta replacing anterior rectus sheath in a dog 3 mos after its insertion. It shows dense fibrous tissue layers on either side of the nylon with lymphocytic infiltration between the threads.

to the nylon filaments and in this neighborhood some foreign body giant cells were also noted.

The other 3 dogs were sacrificed at the end of 6 months. The microscopic findings were similar with the exception that the sheets of fibrous tissue were more dense.

Microscopic Description. The nylon is surrounded by a moderately thick layer of dense collagen which is poorly vascular and relatively acellular. A light residual infiltration of lymphocytes and histiocytes lies next to the fabric and an occasional foreign body giant cell is also encountered.

Rectus Muscle and Anterior and Posterior Rectus Sheath. In this experiment rabbits were used. In 8 animals a defect of about 8 cm. by 1 cm. was made in the anterior and posterior rectus sheath and rectus muscle. The peritoneum was left intact as was the subcutaneous fascia. A rectangular piece of nylon a little smaller than the defect was sutured in as already described. Apart from a mild clinical inflammatory reaction during the first week these animals appeared well and their abdominal walls seemed normal during their whole postoperative period. Five rabbits were sacrificed at the end of 3 months. Microscopically the graft was incorporated in a smooth layer of fibrous tissue. The peritoneum underlying the graft was smooth and there were no adhesions noted between it and the intra-abdominal organs. On microscopy the same fibroblastic reaction with mild lymphocytic and histiocytic inflammatory reaction was seen. There were rare giant cells immediately adjacent to some of the nylon threads but this was not nearly so marked as the foreign body reaction found around the sutures used to fix the graft to the tissue. The remaining 3 rabbits were sacrificed at the end of 6 months. The macroscopic findings were similar with the exception that the sheet of fibrous tissue incorporating the nylon mesh appeared to be a little denser. The lymphocytic and histiocytic infiltration appeared to be less as if the inflammatory reaction were dying down.

Peritoneum only. Eight dogs and 8 rabbits were used. A rectangular piece of peritoneum averaging 8 cm. by 4 cm. was dissected and cut off and a piece of nylon of the same size was substituted.

The postoperative course was satisfactory. The incisions healed well. The nylon was removed after 3 months in 5 dogs and 5 rabbits. Some adhesions were present between omentum and graft but they were easily separated. The nylon was slightly wrinkled and covered by 2 layers of fibrous tissue. The inner layer was like the peritoneum and in some animals it was difficult to recognize the demarcation line between the new and the old peritoneum. In some animals there was a formation of new blood vessels in the new tissue. The suture line in some animals was completely covered by serosal cells.

On microscopic examination there was a thick layer of dense fibrosis on either side of the graft and there was also a moderate infiltration of lymphocytes and histiocytes with some foreign body giant cells.

In 2 rabbits small foci of metastatic cartilage were found. Some of these were calcified and some were converted into osseous tissue.

In 3 dogs and 3 rabbits the grafts were removed after 6 months. The findings were the same as those at 3 months.

On microscopic examination the findings were the same as those at 3 months except that the inflammatory reaction was less marked. In 1 rabbit

Nylon Taffeta Repair of Abdominal Wall Defects with Results

		(GRAFT) REMOVED		RESULTS
		AT 3 MOS	AT 6 MOS	
1st Series - Dogs	Dogs	5	3	Satisfactory
2nd Series - Rabbits	Rabbits	5	3	Satisfactory
3rd Series - Dogs	Dogs	5	3	Satisfactory
4th Series - Rabbits	Rabbits	5	3	Satisfactory

The animals were treated with tincture of iodine 2% per cent prior to the operation. The majority of cases icromycin was used but in a few an reptomycin mixture was used.

Anterior Rectus Sheath Only In 8 healthy dogs a rectangular piece of the anterior rectus sheath was excised. The defect was then repaired with a piece of nylon taffeta a little smaller than the defect so that it was under tension. The graft was sutured to the fascial sheath using interrupted sutures. The animals made an uneventful postoperative recovery. Moderate edema was noted in all cases in the area of the operation 10 days. This quickly settled but in 3 of the 8 dogs some edema appeared to persist in this area. In the other dogs the abdominal wall was quite normal. After 3 months 5 of the dogs were sacrificed. At the 3 dogs which showed some residual inflammatory reaction the grafts were dissected out and subjected to histologic examination. Macroscopically the graft was found to be incorporated in a sheet of tissue which was well united with the surrounding anterior rectus sheath. In 2 of the dogs that had shown clinically some persistent inflammation purulent exudate was found around the nylon but this had cleared up with the production of a strong sheet of fibrous tissue. On the other hand the nylon mesh was surrounded by a narrow sheet of dense fibrous tissue which was infiltrated with a small number of lymphocytes and macrophages. This infiltration was most marked immediately adjacent

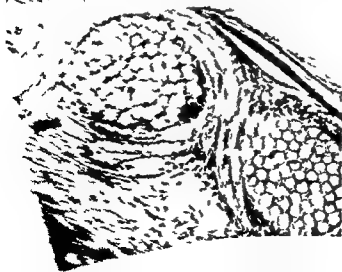


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to the nylon filaments and in this neighborhood some foreign body giant cells were also noted.

The other 3 dogs were sacrificed at the end of 6 months. The microscopic findings were similar with the exception that the sheets of fibrous tissue were more dense.

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Peritoneum only. Light dogs and 8 rabbits were used. A rectangular piece of peritoneum averaging 8 cm. by 1 cm. was dissected and cut off and a piece of nylon of the same size was substituted.

The postoperative course was satisfactory. The incisions healed well. The nylon was removed after 3 months in 5 dogs and 5 rabbits. Some adhesions were present between omentum and graft but they were easily separated. The nylon was slightly wrinkled and covered by 2 layers of fibrous tissue. The inner layer was like the peritoneum and in some animals it was difficult to recognize the demarcation line between the new and the old peritoneum. In some animals there was a formation of new blood vessels in the new tissue. The suture line in some animals was completely covered by serosal cells.

On microscopic examination there was a thick layer of dense fibrosis on either side of the graft and there was also a moderate infiltration of lymphocytes and histiocytes with some foreign body giant cells.

In 2 rabbits small foci of metaplastic cartilage were found. Some of these were calcified and some were converted into osseous tissue.

In 3 dogs and 3 rabbits the grafts were removed after 6 months. The findings were the same as those at 3 months.

On microscopic examination the findings were the same as those at 3 months except that the inflammatory reaction was less marked. In 1 rabbit

the nylon graft was heavily calcified. The calcium was deposited in the fibrous tissue immediately adjacent to the nylon. Here it formed large solid plaques but on occasion there was a network of calcium in the interstices between the individual nylon filaments.

CONCLUSIONS

Nylon taffeta is a satisfactory material for use in replacing defects in the muscle, aponeurosis and peritoneal layers of the abdominal wall.

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EVALUATION OF DACRON SUTURE MATERIAL FOR GENERAL SURGERY*

JOSEPH K. NARAT, JOSEPH P. CANGELOSI AND JOHN V. BELMONT

Evaluation of dacron suture material† was undertaken to continue our studies on synthetic suture materials.^{1,2} Dacron is a polymer, namely a condensation product of ethylene glycol and dimethyl terephthalate. Dacron polyester fiber is not derived from tar nor does it contain tar.

The tensile strength of various gauges of non sterile as well as autoclaved dacron on straight pull dry knot and wet knot pull was compared with that of cotton and silk. Some of the results recorded in Table 1 show that dacron is stronger than cotton or silk. For instance in one series of experiments the dry knot tensile strength of dacron size 10 was 57 per cent greater than that of the corresponding gauge of cotton. Dacron size 30 was 61 per cent stronger and dacron size 20 was 27 per cent stronger than the same sizes of cotton. The tensile strength of dacron was also slightly greater than that of silk. It was found that the tensile strength of wet dacron is identical with that of dry material.

Determination of the frictional coefficient an important factor in the knot holding property of suture materials gave the following results: dacron 0.58, silk 0.52, nylon 0.47 and cotton 0.22. In other words the fractional coefficient of dacron was 2.6 times as great as that of cotton.

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†Furnished by Ethicon, Inc., New Brunswick, N. J.

Table 1. Comparison of Sterile Dacron Cotton and Silk

	SILK	DACRON	COTTON	SILK	REMARKS
					Dacron stronger than cotton
Knot Tensile Strength in lb	20 30 40	100 351 225	50 20 102	11 28 21	27% 61% 17%
Frictional Coefficient		0.18	0.2	0.22	Frict coeff of Dacron 26 times as great as that of cotton
Waxed		no	yes	yes	
Electron Sterilization		applicable	not applicable	not applicable	
Weakened by Autoclaving		no	yes	yes	

Contrary to cotton repeated autoclaving does not reduce the tensile strength of dacron. For example one batch of dacron autoclaved 7 times did not show any diminution in tensile strength.

Dacron thread does not fry. The material is furnished unwaxed while commercial cotton contains wax. This is of importance because acute tissue reactions to waxes derived from spool cotton followed by formation of granulomas have been described by Rosenberg *et al*.³

Electron sterilization of suture materials which is rapidly gaining popularity is applicable to dacron but not to cotton which disintegrates under the influence of rays. There is no loss in tensile strength of dacron sutures when irradiated at 3 million reps.*

To study tissue reaction to dacron strands of dacron cotton silk and catgut were implanted into the abdominal wall of rabbits and biopsies were obtained at various periods after the procedures. The histologic examination showed the intensity of tissue reaction to dacron to be approximately equal to that following insertion of other suture materials.

To test the behavior of dacron *in vivo* the following experiment was devised. Under aseptic conditions the abdominal wall of rabbits was incised and the peritoneum and fascia were closed with either interrupted or continuous sutures of various gauges of dacron cotton silk or linen respectively. At various periods after this procedure the animals were sacrificed, the entire abdominal wall was excised and placed like a diaphragm over a round wooden box which had 2 adapters, one connected with a sphygmomanometer and the other with a compressed air outlet. This arrangement made it possible to inflate the interior of the box to 250 mm Hg which is the highest intra-abdominal pressure recorded in man. The inflation and deflation were repeated 100 times to imitate conditions created by gagging, sneezing, vomiting, coughing or defecation. Numerous experiments showed that during the critical period (namely from the third to the fifth postoperative day (when the strength of the wound is at its lowest) dacron gauge 20 or 30 was able to withstand a pressure of from 200 to 250 mm Hg.

*Abbreviation of roentgen equivalent physical unit

We have used dacron in 89 operations such as thyroidectomy cholecystectomy intestinal anastomosis and herniorrhaphy without any untoward results. No extrusion or formation of granuloma occurred.

SUMMARY

The tensile strength and frictional coefficient of dacron are superior to those of cotton and silk. Dacron does not fray, is not affected by repeated autoclaving, contains no wax, and is well tolerated by tissues. Therefore the statement seems justified that dacron deserves an important place among nonabsorbent suture materials.

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THE REPAIR OF DEFECTS IN THE DIAPHRAGM OF DOGS USING SHEETS OF IVALON SPONGE AND FRESH HOMOLOGOUS GRAFTS OF FASCIA LATA*

IRVING G. PESEK, ARNE E. SCHLAIRER AND JOHN L. KEELEY

Prior work in the repair of defects in the diaphragm of dogs using fresh homologous grafts of fascia lata stimulated a study in which sheets of polyvinyl sponge (Ivalon) were used to correct similar defects. This synthetic material is made from formed polyvinyl alcohol hardened with formaldehyde. It is white and its cut surface has the porous appearance of bread. When dry it is somewhat firm and rigid but becomes soft and elastic when moistened. It has a remarkable affinity for water and except for slight shrinkage is unchanged by temperatures reaching 120°C. Further, polyvinyl sponge appeared to possess the desirable qualities for a synthetic substance useful in the repair of hernia. Grindlay and Waugh¹ on the basis of their experience with polyvinyl sponge suggested that the affinity of polyvinyl sponge for water gave it a kinship with living tissue in which cells exist in an environment of water with fibrous tissue eventually invading and surrounding the polyvinyl sponge, thus in a sense the inert becomes living. Polyvinyl sponge has been used in the successful repair of created defects in the abdominal wall of dogs and as subcutaneous prostheses.^{2, 3, 4} Under these conditions it is possible that the abdominal wall or the skin and underlying

From the Department of Surgery, Stritch School of Medicine of Loyola University, Chicago, Illinois.

tissue afforded a supportive framework and perhaps more effective contact with other tissues from which fibroblasts would grow than in the diaphragm where the insert would be subjected to more deforming forces and therefore tested more vigorously

METHOD

Twenty dogs weighing from 7.2 to 12.3 kg were used. Anesthesia was provided by intra-peritoneal nembutal and respiration was maintained by insufflation of room air through an endotracheal tube. In all instances the dogs received intravenous dextrose during the operative procedure. The left hemithorax was entered through a laterally placed incision in the lower chest wall. Approximately $\frac{1}{4}$ to $\frac{1}{2}$ of the muscular portion of the left hemidiaphragm was excised. Sheets of Ivalon 3 to 1 mm thick previously sterilized by boiling in tap water for 30 min. were sutured to the peritoneal side of the diaphragmatic defect. The sponge implant was secured by means of interrupted and continuous sutures of 4/0 cotton achieving an imbrication of 1 cm. The chest wall was then closed in layers with cotton sutures and without drains. No intrapleural antibiotics were used; however the dogs received daily injections of 300,000 units of penicillin for the first 3 days after operation. Later in the study roentgenographic examinations were made after pneumoperitoneum was induced for better visualization of the diaphragmatic contours. Animals were sacrificed at intervals of 1½, 2, 4, 6 and 12 mo. at which time gross and microscopic studies were made.

RESULTS

In the immediate postoperative period apparently due to anesthesia 2 dogs were lost in the first 24 hr. The rest of the animals began to be active about 3 to 5 days after operation. The x-ray studies made at intervals before sacrifice showed normal diaphragmatic contours and lung fields (Fig. 1). At autopsy there were firm adhesions between the sponge insert and the lung, stomach, liver, and omentum. In no instances were any collections of encapsulated fluid noted about the sponge. In the animals sacrificed after 12 mo. the sponge insert was firm and it was difficult to identify upon viewing the gross specimen (Fig. 2).



Fig. 1 Roentgenogram showing diaphragmatic contour in dog 6 mos after implant of sponge.

We have used dacron in 89 operations such as thyroidectomy cholecystectomy intestinal anastomosis and herniorrhaphy without any untoward results. No extrusion or formation of granuloma occurred.

SUMMARY

The tensile strength and frictional coefficient of dacron are superior to those of cotton and silk. Dacron does not fray, is not affected by repeated autoclaving, contains no wax, and is well tolerated by tissues. Therefore the statement seems justified that dacron deserves an important place among nonabsorbent suture materials.

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THE REPAIR OF DEFECTS IN THE DIAPHRAGM OF DOGS USING SHEETS OF IVALON SPONGE AND FRESH HOMOLOGOUS GRAFTS OF FASCIA LATA*

IRVING G. PESEK, ARNE E. SCHAIRER AND JOHN L. KEELEY

Prior work in the repair of defects in the diaphragm of dogs using fresh homologous grafts of fascia lata stimulated a study in which sheets of polyvinyl sponge (Ivalon) were used to correct similar defects. This synthetic material is made from foamed polyvinyl alcohol hardened with formaldehyde. It is white and its cut surface has the porous appearance of bread. When dry it is somewhat firm and rigid but becomes soft and elastic when moistened. It has a remarkable affinity for water and except for slight shrinkage is unchanged by temperatures reaching 120°C. Further, polyvinyl sponge appeared to possess the desirable qualities for a synthetic substance useful in the repair of hernia. Grindlay and Waugh¹ on the basis of their experience with polyvinyl sponge suggested that the affinity of polyvinyl sponge for water gave it a kinship with living tissue in which cells exist in an environment of water with fibrous tissue eventually invading and surrounding the polyvinyl sponge; thus in a sense the inert becomes living. Polyvinyl sponge has been used in the successful repair of created defects in the abdominal wall of dogs and as subcutaneous prostheses.^{2, 3, 4} Under these conditions it is possible that the abdominal wall or the skin and underlying

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Fig 1 Photograph showing diaphragm of dog after repair with homologous fascia lata



was replaced by a layer resembling granulation tissue. Finally the graft was represented by a sheet of dense fibrous tissue which appeared to be the supporting structure. Only fragments of the original graft could be identified microscopically. It appears that homologous fascia lata grafts are satisfactory in the repair of diaphragmatic defects during the period of observation. However they do not survive intact as autogenous fascia lata grafts are reported to do when used in the same manner but observed for a shorter period of time.

CONCLUSIONS

In 2 series of animals approximately 60 per cent of the central portion of the left diaphragm was excised. Repair of these defects was accomplished by use of Ivalon sheet in one series and fresh homologous fascia lata grafts in the other. Both materials appear to be effective in the repair of defects thus created and observed for periods up to 12 mo.

From the preliminary examinations of the microscopic sections in these studies both polyvinyl sponge and fascia lata form a framework about which a sheet of fibrous tissue grows. The sponge appears to undergo fragmentation while the fascia lata appears to undergo absorption and replacement.

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Fig 2 Photograph showing diaphragm of dog removed 12 mos after implant of sponge

Microscopically the findings which we noted were similar to those previously reported by Crindley and Waugh.¹ The sections showed spaces irregular in size and arrangement filled with a faint blue material after staining with hematoxylin and eosin. Higher magnification disclosed that these pale blue areas had a porous bubble like appearance. Interspersed between these spaces were infiltrations of collagen fibers containing spaces lined by a single layer of cells which may be vascular endothelium as has been suggested or may represent atypical flattened fibroblasts. In the specimens studied 1 1/2 and 2 mo after implantation there was a marked but loose fibrous ingrowth around the sponge and no inflammatory reaction was noted. At the 4 mo interval the sponge appeared to be somewhat more compact and the connective tissue was more dense but sparsely cellular. Later at the 6 mo interval the sponge trabecular appear fragmented with very abundant foreign body giant cell reaction. At the 12 mo interval the trabecular fragmentation was still noted with considerably less trabeculae present and more cellular fibers. It becomes apparent then that for some reason a foreign body giant cell reaction is provoked sometime during the interval of 4 to 6 mo after implantation. We do not know the significance of this reaction but subsequent studies may show that this represents a critical period in the assimilation of the graft. We feel that this should stimulate a further study of this giant cell reaction.

In another series of experiments under essentially the same conditions of anesthesia exposure and technique as described in the use of Ivalon sheets but without antibiotics fresh homologous grafts of fascia lata obtained from a dog's thigh were used to repair diaphragmatic defects of similar size. All animals survived and were observed for periods up to 12 mo. X-ray studies showed no disturbance in the contour of the diaphragm and no evidence of herniation. The animals were likewise sacrificed at intervals and gross examination disclosed completely satisfactory repair of the defect in 13 dogs (Fig 3). In 1 dog the graft was thin and evagination involving about 60 per cent of it occurred. Multiple microscopic sections of the grafts taken at necropsy showed in early inflammatory reaction on both surfaces of the entire graft as indicated by edema and infiltration with polymorphonuclear leukocytes lymphocytes and plasma cells. Later the inflammatory reaction

In this first phase of the study, the spontaneous respiratory efforts of the patient on the ventilator were abolished by deepening the level of anesthesia. In subsequent studies this will be achieved by hyperventilating the individual.

Ether was the basic anesthetic agent employed in almost all cases.

RESULTS

To date 30 patients have been studied, 15 with the ventilator and 15 without. The averaged values for the 2 groups are shown in Table 1.

Arterial Oxygen Saturation The average blood oxygen saturation was depressed in both groups immediately prior to operation, a finding which later proved to be due largely to the effect of relatively heavy pre-anesthetic medication which included morphine or demerol and a barbiturate. Samples taken in subsequent patients prior to the pre-anesthetic medication or when only atropine was given were generally within normal limits. This moderately diminished preoperative arterial oxygen saturation was accompanied, as noted below, by a mild increase in total carbon dioxide content of arterial blood. However, this increase was not sufficient to alter the blood pH to a degree detectable with the pH meter.

In general, the degree of arterial oxygen saturation, while somewhat below normal in both groups, was perhaps more uniform in the respirator group, yet this average difference was not great. This was so even though the respirator group was carried at a depth of anesthesia sufficient to halt all respiratory efforts on the part of the patient. Isolated instances of hypoxia occurred in both groups, but it was only in the group in which the Jefferson ventilator was not used that severe cardiac depression due to poor ventilation occurred. Virtual cardiac arrest intervened in 1 patient and profound shock in the other—both of whom responded to vigorous and rapid manual compression of the gas bag. Of course, hypoxia may result from oligemic shock as well as other causes.

Total Arterial CO₂ Content Whereas during the control period the average total carbon dioxide content of arterial blood tended to be elevated (likely due to the heavy pre-anesthetic medication in the earlier patients), there was a gradual average decline in this value during the period of anesthesia.

Table 1 Cumulative Average Values (Arterial Blood) 15 Patients with Jefferson Ventilator and 15 Without

VENTILATOR		AFTER ENDOTRACHEAL TUBE						RECOVERY ROOM	DAY AFTER
		C	1 10 min	2 20 min	3 40 min	4 70 min	5 130 min		
WITH	pH	7.48	7.45	7.44	7.49	7.50	7.54	7.47	7.49
WITHOUT		7.44	7.43	7.41	7.42	7.42	7.44	7.37	7.46
WITH	Total CO Content	48.55	48.07	47.12	47.21	45.97	42.76	46.76	49.47
WITHOUT		46.24	44.5	43.87	47.81	47.36	42.74	47.70	47.01
WITH	Per cent Oxygen Saturation	81.12	91.43	90.82	85.21	84.46	84.25	82.90	82.74
WITHOUT		87.1	93.9	91.8	97.8	91.4	82.3	88.0	86.5

* The relatively depressed control levels were found to be due in part to the effect of the pre-anesthetic medication.

Anesthesia and Pulmonary Surgery

ANESTHESIA RESEARCH I USUAL vs MECHANICAL METHOD OF INFLATION*

JAMES D HARDY JOHN O DAMPEER JR M DON TURNER AND
GLACE BITTENBENDER

Cardiac arrest due to defective anesthetic management constitutes a major source of operative mortality. Moreover, inadequate pulmonary ventilation ranks high as a specific example of defective anesthesia. When the minute gas exchange is below a critical level for the patient in question, the potentially lethal conditions of carbon dioxide retention with acidosis and of anoxia result. Many workers have shown (and countless surgeons have suspected from the color of the blood) that anoxemia and hypercapnia usually have existed for some time before the heart succumbs. One facet of the problem would appear to be the need for an increased awareness on the part of many anesthetists as to what adequate ventilation entails. For anesthetists with sound basic training in cardiorespiratory physiology rarely allow the patient to become anoxic or hypercapnic. This is perhaps but another way of stating that there exists a shortage of well trained anesthetists. However, it occurred to us, as it had to others, that a mechanical device which could be set to ventilate the patient at a definite minute rate and volume might enhance the efficiency of pulmonary ventilation if properly employed. For this reason we have conducted a clinical comparison between usual intermittent manual assistance and the Jefferson ventilator.

METHOD

Prior to operation a specially prepared No. 17 needle was inserted into a femoral artery. Control and subsequent intermittent arterial blood samples were drawn throughout the period of anesthesia and during recovery therefrom. The following day a final arterial blood sample was drawn. Blood oxygen and total carbon dioxide analyses were made with the Van Slyke manometric blood gas apparatus and blood pH measurements with a Beckman pH meter.

Mechanical inflation was used in approximately one half the patients studied. As soon as the endotracheal tube had been inserted the ventilator was switched into the system to inflate the lungs at a fixed rate and volume employing both positive and negative phases. The degree of inflation was regulated by direct inspection of the lung in patients having open chest operations and by clinical judgment in those having abdominal procedures. The clinical judgment of the anesthetist was important even when using the machine, therefore, since the ventilator was regulated by this individual.

*Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi. This work performed under Army Contract No. DA 49-007 MD 627, Office of the Surgeon General, Department of the Army.

With the technical assistance of Virginia Ward and Thelma Carter.

THE EFFECT OF ETHER ANESTHESIA ON PYRUVATE METABOLISM*

WILLIAM R. DRUCKER CHRISTINE COSLEY ROBERT STUITS
MAX MÜLLER JAMES W. CRAIG AND HIRSH WOODWARD

Reynoso¹ reported in 1853 that glycosuria followed ether anesthesia in man. It was not until after the turn of the century however that a report was made relating an increase in blood sugar to ether anesthesia.² In the following 50 years considerable attention was directed toward the disturbance in carbohydrate metabolism associated with anesthesia because of the possibility that inhibition of cellular carbohydrate metabolism is responsible for ether narcosis. This work has been reviewed by Gerrod.³

Previous studies done in our laboratory concerned the effect of ether anesthesia in man on the metabolism of two hexoses glucose and fructose.⁴ It was found that ether caused a marked disturbance in the tolerance for glucose but relatively little disturbance in the tolerance for fructose. The result was interpreted as indicating that ether inhibits specific enzymatic processes.

The next step in the study of the effect of ether on carbohydrate metabolism in humans was to determine the effect of ether on pyruvate metabolism. This seemed logical because pyruvic acid is the final product in the anaerobic scheme of glycolysis and it is converted into acetyl CoA which supplies fuel for the citric acid energy producing cycle. Also many of the studies on tissue slices have indicated that narcotics may act on the metabolism of pyruvate.

METHOD

Five patients hospitalized for rehabilitation at Highland View Cuyahoga County Hospital volunteered for this study. Each patient received 2 pyruvate tolerance tests, 1 a day or 2 prior to anesthesia as a control and 1 during the administration of anesthesia. Supplementary vitamin B complex was given for a few days prior to the study in doses that were 10 to 60 times the normal requirement. The subjects were fasted and required to remain in bed on the day of the tests. Blood samples drawn through a Courmand needle were obtained prior to the start of anesthesia and subsequently every 30 min during the 90 min of anesthesia and the 2 hr following the cessation of ether administration. Anesthesia was induced by the intravenous administration of 150 to 300 mg of thiopental sodium. Ether anesthesia was started with a mixture of nitrous oxide oxygen and ether and maintained with endotracheal oxygen ether. Thiopental sodium was used to minimize the excitement frequently encountered during the induction of ether narcosis. All anesthetics were given by the same person (CC). Thirty minutes after surgical anesthesia was obtained a specimen of blood was drawn and an infusion of sodium pyruvate was started. The infusion was given at a con-

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With the technical assistance of Nancy Hofmann Helen Bassett Naomi Nickerson and Elsie Mirek.

The authors express their appreciation to Warren Strauss for his assistance with the illustrations.

thetia, whether the Jefferson ventilator was or was not used. These values regained control or normal levels while the patients were under post anesthetic observation in the recovery ward. Thus it would appear from the data that on the average a degree of hyperventilation was produced in both groups.

Arterial pH. The average values for the 2 groups of patients were similar but there was some divergence of the pH toward the alkaline side in the ventilator group and toward the acid side in the usual group. In isolated patients particularly in the usual group significant acidosis was encountered.

DISCUSSION

By averaging the values in each group one achieves generalization and a certain balance in the evaluation of the data. Satisfactory ventilation can be achieved by either method. At the same time, highly significant exceptions—the isolated patients in whom anesthesia was not satisfactory—are obscured by averages. Let it suffice to state here that instances of seriously defective anesthesia occurred primarily in the group in which the ventilator was not employed. Moreover as the study progressed and the purpose of the arterial blood sampling became known to the personnel, there was a tendency for the nurse anesthetists to utilize more manual assistance and to diminish the incidence of defective ventilation in the usual group.

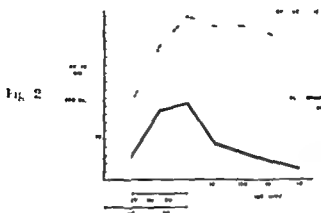
SUMMARY AND CONCLUSIONS

1 Thirty patients undergoing general anesthesia have been studied with serial determinations of the oxygen saturation, pH and total CO_2 content of arterial blood. In approximately one half, the usual spontaneous respiration with or without indicated manual assistance was employed. In the other half, the Jefferson ventilator was switched into the system as soon as the endotracheal tube was in place. Ether was the basic anesthetic agent in virtually all cases.

2 The use of an atropine-morphine-barbiturate pre-anesthetic medication rendered pre-anesthetic control levels of arterial oxygen saturation somewhat below normal. In the earlier patients studied, control arterial total CO_2 content was mildly increased. In subsequent subjects the control sample was drawn before the pre-anesthetic medication had been given and furthermore the dosage of such medication was reduced.

3 In general the arterial pH, total CO_2 content and oxygen saturation were comparable in both groups. However serious and clinically obvious anesthetic difficulties were not encountered in the group in which the Jefferson ventilator was used, whereas 2 such instances occurred in the group in which it was not used. No ill effects were observed from the ventilator.

4 It is concluded that while efficient management can be conducted without an artificial ventilator, the judicious use of such an aid adds to the safety factor in avoiding hypoventilation.

LACTIC ACID & LVED DURING PYRUVATE TOLERANCE TESTS
BEFORE & DURING ETHER ANESTHESIA

out ether are illustrated in Figure 2. The lactic acid rise during the first 30 min. of pyruvate infusion averaged 5.5 mg/100 ml. for the control tests and 14.5 mg/100 ml. for the anesthesia tests. The differences between the 2 tests became progressively more pronounced with time. Two hours after discontinuation of the infusion and anesthesia the lactic acid level remained 12.6 mg/100 ml. above the fasting level and 5.6 mg/100 ml. above the level obtained at the time the infusion was started (30 min. after the start of anesthesia).

Glucose. The mean rise of fasting blood glucose during the first 30 min. of anesthesia was 39 mg/100 ml. When the pyruvate infusion was started the glucose level fell in both the control and anesthesia tests.

DISCUSSION

Pyruvic acid stands at the cross roads of protein, fat and carbohydrate metabolism (Fig. 3). Pyruvate may be converted into protein, fat, glycogen, lactate, oxaloacetate or oxidized *via* acetyl CoA into the citric acid cycle to yield energy. There may also be pathways of pyruvate metabolism presently unknown. It is not possible to discern from the present study which of these many pathways of pyruvic acid metabolism is blocked by ether anesthesia. Considerable work on tissue slices, however, suggests that oxidative decarboxylation of pyruvate is blocked, probably at some point in the cytochrome system.

Decarboxylation of pyruvic acid requires 4 interdependent factors. In this

PATHWAYS OF PYRUVATE METABOLISM

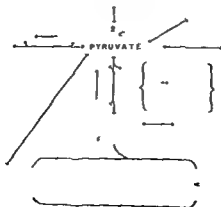


Fig. 3

stant rate with an infusion pump for 1 hr at the end of which time both the infusion and the ether were discontinued. Capillary oxygen content was followed at frequent intervals by an oximeter. Samples of arterial blood were drawn for analysis of oxygen saturation prior to the start of anesthesia and 30 and 90 min after surgical anesthesia was reached. An electrocardiogram was obtained on the day prior to the test and frequent recordings were made during the period of narcosis.

Crystalline sodium pyruvate* dissolved in pyrogen free water† was filtered through a Seitz Filter at 10 mm Hg suction pressure. The solution was then diluted to contain 16.5 gm of sodium pyruvate/l of solution (13.2 gm pyruvic acid/l). The pH was adjusted to 7.40 by the addition of 0.45 ml of 0.1 N HCL/L. Each subject was given 9.09 ml (0.12 gm)/kg of body weight over a 1 hr period. This dose was found by us to produce a rise in blood pyruvic acid of 2.3 mg/100 ml in non diabetic hospitalized individuals. All solutions were prepared within 2 days of use and were refrigerated until the time of administration. No reactions were encountered with the use of this solution.

The chemical methods for the determination of glucose and pyruvate have been previously described.⁶ Lactic acid was determined by the method of Barker and Summerson.⁷

RESULTS

Pyruvate There was no significant change in the fasting pyruvic acid level during 30 min of anesthesia prior to the start of the pyruvate infusion (Fig. 1). The average rise in pyruvic acid during the first 30 min of pyruvate infusion was 1.33 mg/100 ml for the control and 3.13 mg/100 ml for the anesthesia tests. At the end of 60 min infusion the mean rise above fasting was 3.32 mg/100 ml for the control tests and 2.61 mg/100 ml for the anesthesia tests. When the infusion was stopped there was a prompt return to the fasting level in the control tests whereas in the anesthesia study the pyruvic acid was still 0.55 mg/100 ml above the fasting level 2 hr after the infusion and ether were discontinued. The differences between the tests at 60, 150, 180 and 210 min have all proved to be significant by application of the Fisher t test.

Lactate When pyruvate is given there is a rise in blood lactic acid. The changes in lactic acid during the administration of pyruvate with and with

PYRUVATE TOLERANCE BEFORE AND DURING ETHER ANESTHESIA

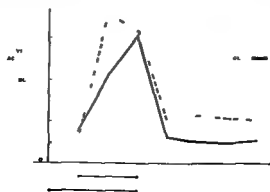


Fig. 1

*Nutritional Biochemical Company of Cleveland

†Courtesy of Meade Johnson Company

THE EFFECT OF ANESTHESIA ON BLOOD VOLUME AND OSMOTIC PRESSURE *

WILLIAM R. DRICKER, R. STEVEN C. COSTLEY, J. DOCKERY
AND WILLIAM D. HOLDEN

Homeostasis in living organisms depends to a large extent on the maintenance of physiological osmotic pressures. The distribution of water and permeable solutes throughout plants and animals depends on osmotic effects. Many of the disorders in body fluid metabolism are explainable on the basis of changes in osmotic pressure.

In 1955 Moore and his associates reported that the osmotic pressure of plasma increased during the course of ether anesthesia. It is known that ether causes many physiologic disturbances. Some of these such as decreased renal excretion of water may be a response to an increase in the osmotic pressure of plasma. It seemed desirable therefore to determine the basis for the rise in osmotic pressure during ether anesthesia and whether it could be correlated with any measurable physiologic change.

METHOD

Ten patients hospitalized for rehabilitation at Highland View Cuyahoga County Hospital volunteered to undergo 90 min of ether anesthesia without surgery. All patients received demerol 50 mg and atropine 0.64 mg as pre-anesthetic medication. Samples of venous blood were drawn through a Courmand needle placed in a forearm vein. The first sample was drawn prior to the start of anesthesia. Subsequent samples were obtained every 30 min during anesthesia and for 2 hr after discontinuation of anesthesia.

The anesthesia was induced by intravenous administration of 150 to 300 mg of thiopental sodium. Ether anesthesia was started with a mixture of nitrous oxide-oxygen and ether and maintained with endotracheal oxygen-ether. All anesthetics were administered by the same person (C.C.). During the final 60 min of ether each subject received an intravenous infusion of sodium pyruvate or sodium lactate by means of a constant rate infusion pump as part of another study. During anesthesia capillary oxygenation was checked by an ear oximeter. Blood and plasma volume, hematocrit, plasma sodium concentration and blood oxygen saturation were determined prior to the start of anesthesia and 30 and 90 min after surgical anesthesia was reached. An electrocardiogram was obtained the day before each study and frequently during the course of anesthesia. Blood pressures were obtained by a sphygmomanometer. Urine was collected from 7 patients every half hour by an indwelling catheter and the urine was measured for volume and sodium concentration.

The blood for osmolality determinations was placed in refrigerated tubes with dried heparin. The tubes were centrifuged and the plasma pipetted into a small tube which was corked and stored at -11°C . This was done to

*From the Departments of Surgery, Western Reserve University School of Medicine, Highland View Hospital and University Hospitals of Cleveland, Cleveland, Ohio. Work supported in part by U.S.P.H.S. grant number A 1253.

With the technical assistance of Nancy Hofmann and Helen Bassett and the assistance of Warren Strauss with the illustrations.

mine 2, lipoic acid 3 magnesium or manganese and 1 DPN (diphosphopyridine nucleotide) (Fig 3) It is unlikely that thiamine deficiency was responsible for the disturbed pyruvate metabolism since these patients received nutritionally adequate diets with large supplementary doses of vitamin B complex and had normal blood thiamine levels with no clinical evidence of avitaminosis There is insufficient evidence to allow speculation on the relationship of lipoic acid and magnesium or manganese to anesthesia

The fourth factor in the oxidative decarboxylation of pyruvic acid to acetyl CoA is oxidized DPN Should there be some interference in the cytochrome system which would inhibit the reformation of DPN-ox from DPN-H increased quantities of pyruvate might be reduced to lactate The reduction of pyruvate to lactate would form DPN-ox The renewed supply of DPN-ox would allow conversion of more pyruvate to acetyl CoA The increased rise in lactate found in this study is compatible with this concept

The cause of the interference with the reformation of DPN-ox remains to be determined Anoxia *per se* is an unlikely cause since all of the patients had normal blood oxygen saturation during anesthesia Also studies on tissue slices have shown that narcotics in therapeutic doses do not interfere with the uptake and activation of oxygen by living cells⁸ Quastel⁵ has postulated that narcotics produce a block between DPN and cytochrome

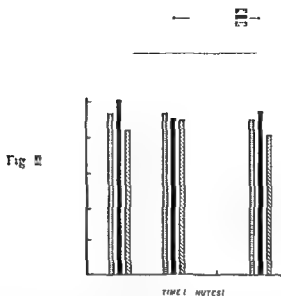
In summary, it cannot be stated on the basis of this study where the block occurs in pyruvate metabolism during ether anesthesia Possible mechanisms have been discussed It is known that cells depend on the combustion of pyruvate in the citric acid cycle for most of their energy It is tempting to speculate therefore that the state of ether narcosis in humans may be due to decreased cell energy as a result of impaired utilization of pyruvate

CONCLUSIONS

- 1 Tolerance for exogenous sodium pyruvate is diminished in humans during ether anesthesia
- 2 The site of the block in pyruvate metabolism in anesthetized humans must await further study for elucidation
- 3 The impaired metabolism of pyruvate during ether anesthesia may be sufficient to diminish the energy production necessary for functional activity of neural cells This may be sufficient to induce the state of anesthesia

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PLASMA SODIUM CONCENTRATION DURING
ETHER ANESTHESIA

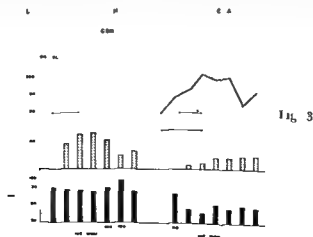
otic pressure. The urinary sodium concentration decreased after the induction of ether anesthesia and remained considerably reduced thereafter.

All patients were awake and able to talk before the termination of the study. There was no change in capillary oxygen saturation by ear oximetric readings. Blood analysis revealed normal oxygen saturation for all cases during anesthesia.

Plasma with an elevated osmotic pressure showed a considerable fall in the osmotic pressure when this plasma was allowed to stand at room temperature or shaken or heated slightly. There was no change in the osmotic pressure of the control plasmas with similar treatment.

DISCUSSION

Effective osmotic pressure is that fraction of the total osmotic pressure which governs movements and distribution of water between cells and extracellular fluid. Sodium contributes the largest component of effective plasma osmotic pressure because of its high concentration in extracellular fluid and its relative exclusion from cells. During anesthesia without associated sur-



PLASMA OSMOTIC PRESSURE, HEMATOCRIT, AND BLOOD
VOLUME DURING ETHER ANESTHESIA

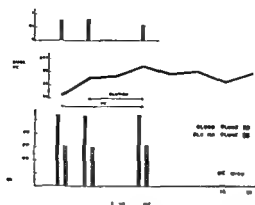


Fig 1

prevent the volatilization of ether from the plasma. The osmolarity determinations were subsequently performed by the freezing point depression method with the Fiske osmometer.

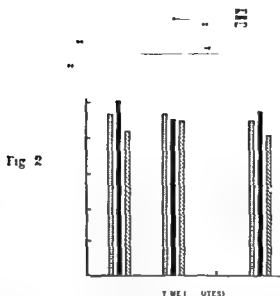
Determinations of blood and plasma volume were done by the method described by Storassli *et al*¹ using 131 I tagged human albumin. Arterial hematocrits were determined by the method of Wintrobe. Plasma and urine sodium concentrations were obtained by the use of a Beckman flame photometer and oxygen was determined by a Van Slyke manometric gas analysis apparatus.

RESULTS

The average values of the venous osmolarities determined every 30 min during the course of this study are illustrated in Figure 1. There was a rise of 13 mOsm during the first 30 min and an additional mean rise of 9 mOsm during the final 60 min of ether anesthesia. Measurements made during the 2 hr after ether was discontinued demonstrated a fluctuating but persistently elevated plasma osmotic pressure.

In contrast to the 8 per cent rise in plasma osmolarity with ether there were no significant changes in blood volume, plasma volume, hematocrit, or plasma sodium. In order to rule out the possibility that administration of sodium containing fluids (98 mEq of Na) prevents a fall in plasma sodium, 5 additional patients were studied who received a 10 per cent solution of sugar in water. The average sodium concentration for these patients is compared with the average sodium concentration for the patients who received sodium lactate or sodium pyruvate (Fig 2). There is no significant difference in the 3 groups. The patients who received sugar solutions showed a rise in osmolarity comparable to the rise found with lactate and pyruvate infusions with ether anesthesia but no change in blood or plasma volume or hematocrit.

Control tests were run a day or two prior to anesthesia for 7 patients in which they were given an amount of fluid similar in volume and sodium concentration to that given during anesthesia. The marked depressant effect of ether on salt and water excretion is illustrated in Figure 3. An interesting observation is that although the urine half hourly volume abruptly decreased during anesthesia, it began to rise again despite a rising plasma os

PLASMA SODIUM CONCENTRATION DURING
ETHER ANESTHESIA

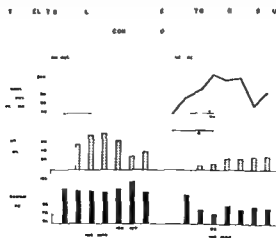
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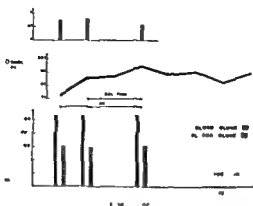


Fig 1

prevent the volatilization of ether from the plasma. The osmolarity determinations were subsequently performed by the freezing point depression method with the Fiske osmometer.

Determinations of blood and plasma volume were done by the method described by Storash *et al*,³ using I^{131} tagged human albumin. Arterial hematocrits were determined by the method of Wintrobe. Plasma and urine sodium concentrations were obtained by the use of a Beckman flame photometer and oxygen was determined by a Van Slyke manometric gas analysis apparatus.

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RESPIRATORY ACIDOSIS AND PULMONARY VENTILATION DURING OPEN THORACOTOMY

The Effect of Compression of the Lung

THOMAS I. NIXON, JR., JOYCE I. PRICE, AND JOHN H. CIBBON, JR.

The respiratory acidosis which occurs during operations is due to inadequate pulmonary ventilation. Such acidosis can be avoided by assisting the respiratory efforts of the patient or, preferably, by ventilating the lungs mechanically with no respiratory effort on the part of the patient. When the chest is opened it has been shown that the pulmonary ventilation must be considerably increased to avoid respiratory acidosis.¹ The following study was undertaken to determine why an increase in pulmonary ventilation was necessary. With an open thoracotomy the increase in total pulmonary ventilation necessary to prevent respiratory acidosis was found to be the result of compression of the lung.

METHOD

Six patients were studied during operations requiring an open thorax (Table 1). Anesthesia was induced with intravenous thiopental sodium and succinylcholine. After introducing an endotracheal tube anesthesia was continued with ether or nitrous oxide and oxygen. The patients were made apneic and pulmonary ventilation was carried on by an intermittent positive and negative pressure ventilator (The Jefferson Ventilator). Apnea was produced by hyperventilation in 5 patients and by succinylcholine in Case #4.

The sampling apparatus and techniques used have been described in detail in a previous publication.² In brief, a mixing chamber and a gas flow meter were incorporated in the expiratory line of the closed rebreathing anesthetic circuit. Thus mixed expired gas samples could be obtained and the ventilation measured. An infra red absorption analyzer gave a continuous indication of the carbon dioxide in the respired gases in the endotracheal tube.³ Using the carbon dioxide analyzer as a monitor, the ventilation was adjusted until the carbon dioxide tension ($p\text{CO}_2$) of the expired gases lay at some point between 35 and 40 mm Hg with 1 exception (Case #4). The ventilation was adjusted by changing the tidal volume. This was accomplished by increasing or decreasing the difference between the positive and negative pressures on the ventilator. A steady state was considered to exist when the $p\text{CO}_2$ of the end expired gas had remained at a constant value for 10 min. with no change in pulmonary ventilation. The pulmonary ventilation was then measured while a sample of arterial blood was drawn. The $p\text{CO}_2$ and O_2 saturation of the arterial blood were determined. These measurements were made (1) after the operation was under way but before the chest was opened, (2) after the chest was opened but before the lung was disturbed, (3) during the intrathoracic portion of the operation which necessitated some compression of the lung and (4) in 3 patients after the lung was re-expanded.

RESULTS

The results are tabulated in Table 1 and illustrated graphically in Case #2 by Figure 1. In all patients the ventilation required to maintain the

gery there is no significant loss of sodium from the body either externally or into a third space. The plasma sodium concentration should therefore remain unaltered providing there is no loss or gain of fluid into the extracellular space. But if some agent were added to the plasma which increases the effective osmotic pressure the extracellular fluid volume should rise due to a transfer of water from the cells. This in turn should be reflected by a fall in the sodium concentration. The fact that neither a measurable rise in blood or plasma volume nor a fall in sodium concentration or hematocrit was found in this study despite a rise of 22 mOsm in plasma osmotic pressure suggests that the increase was not due to a substance which remained confined to the extracellular space.

The renal response to an increase in effective plasma osmolarity is a reduction of urinary volume. If dehydration is also present there is a decreased excretion of sodium as part of the dehydration reaction.² The observed rise in osmolarity during ether anesthesia theoretically could have a causal relationship to the decreased renal excretion of water and sodium. Although a marked reduction in renal excretion of water and sodium was found during anesthesia there was an increase in half hourly urine volumes despite a rising osmotic pressure. This observation also supports the hypothesis that the rise in osmotic pressure was due to a substance distributed across both extra and intracellular compartments.

The fact that the elevated osmotic pressure falls when the plasma either stands at room temperature or is heated slightly is evidence that ether, a highly volatile substance, is responsible for the increase in osmotic pressure.

SUMMARY AND CONCLUSIONS

1 The increase in plasma osmotic pressure with ether anesthesia is probably due to ether incorporated in the plasma.

2 The increase in plasma osmotic pressure is not associated with a measurable alteration of blood or plasma volume, hematocrit or sodium concentration. Therefore the diminished renal excretion of water and sodium during ether anesthesia cannot be explained by the elevated osmolarity of the plasma.

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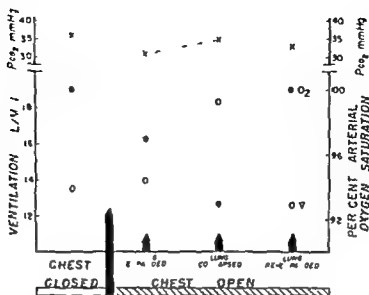


Fig. 1 Graphs of the $p\text{CO}_2$ and per cent saturation with oxygen of arterial blood and the pulmonary ventilation in Case No. 2. The $p\text{CO}_2$ was maintained at approximately the same level throughout. The pulmonary ventilation with the chest open and the lung collapsed had to be markedly increased to avoid a rise in the $p\text{CO}_2$. With the chest open and the lung expanded the pulmonary ventilation could be kept at approximately the same level as when the chest was closed without any rise occurring in the $p\text{CO}_2$. The oxygen saturation fell slightly when the chest was opened and the lung expanded but was still within the normal range. With the lung collapsed despite the increase in ventilation there was a significant decrease in the saturation which rose again to normal levels when the lung was re-expanded with the chest still open.

$p\text{CO}_2$ of the arterial blood at the same level was approximately the same before and after the chest was opened. Compression of the lung however necessitated a considerable increase in ventilation to keep the $p\text{CO}_2$ at the same level with one exception (Case #6). With the lung compressed even with hyperventilation there was in most cases a significant drop in the O_2 saturation of arterial blood. In the 3 instances in which the measurements were repeated after the lung was re-expanded it was again possible to maintain the $p\text{CO}_2$ at the same level with the former and lesser pulmonary ventilation.

DISCUSSION

In these observations an open thoracotomy did not in itself result in the need for increased pulmonary ventilation to avoid a rise in the $p\text{CO}_2$ of arterial blood. Only after the lung was compressed was it necessary to increase the ventilation to prevent the $p\text{CO}_2$ from rising. The smallest increase was required in Case 6 the repair of a hiatal hernia where only slight compression of the lower lobe of the left lung was used.

During compression of the lung some blood will pass through capillaries in the walls of collapsed alveoli. This in effect results in an anatomic shunt of some unaltered venous blood through the pulmonary circulation to the

Table I

CASE	OPERATION	PCO ₂ ARTERIAL BLOOD			VENTILATION			ARTERIAL OXYGEN SATURATION		
		CHEST CLOSED	CHEST OPEN	MM HG LUNG COMPRESSED	CHEST LUNG	CHEST LUNG	L/MIN LUNG	CHEST CLOSED	CHEST OPEN	LUNG COMPRESSED
1	Lobectomy	35	32	32	33	160	156	92	91	90
2	Excision Neurofibroma	36	31	35	33	135	141	100	97	100
3	Excision Aortic Aneurysm	35	38	35		200	190	100	98	95
4	Lobectomy	58	59	65	53	162	158	96	96	97
5	Excision Aortic Aneurysm	39	38	41		171	168	97	98	93
6	Repair Hiatal Hernia	36	34	36		99	116	98	98	92

FAILURE OF THE CIRCULATION IN ACUTE HYPONIA*

IBRAHIM K. DACHIR AND GEORGE H. A. CLOWES, JR.

Experimentally, hypoxia resulted in standstill of the heart when the arterial oxygen content fell below three volumes per cent.¹ On the other hand cardiac arrest from pure hypercapnia did not occur in intact animals.^{1,7}

The present study was conducted in dogs subjected to acute anoxia to determine which portion of the circulation fails first. It is the purpose of this paper to present evidence that the heart failed under these conditions while the peripheral resistance and venomotor tone remained normal or increased.

METHOD

The aortic blood flow (exclusive of coronary circulation) was measured with a Shipley rotameter² that recorded on a Grass Ink writing Oscillograph Model III B⁶ through a demodulator. The blood was directed to the rotameter by way of an Eckstein cannula.³ Simultaneous electroencephalographic and electrocardiographic tracings (lead II) were made on separate channels of the same oscillograph. Left auricular, right auricular and pulmonary artery pressures were measured with water manometers while femoral artery pressure was recorded by a mercury manometer.

The carbon dioxide and oxygen contents of arterial blood samples were determined by the method of Neill and Van Slyke.⁹

The left pleural cavity of 7 mongrel dogs anesthetized with sodium pentobarbital was entered by excising the third and fourth ribs. The thoracic aorta was mobilized from the arch down to the level of the fifth rib and the 2 innominate vessels were freed. The left carotid artery was isolated through a separate incision made in the neck. Coagulation of blood was prevented by the intravenous administration of 5 mg. of heparin per kg. of body weight. The left carotid artery was cannulated and ligated distally. The cannula led to a reservoir placed at a height of 2 m. above the level of the heart. The left pulmonary artery and the left auricle were cannulated through segmental pulmonary vessels. The left femoral artery was cannulated and attached to a mercury manometer. The venous pressure was measured through a catheter placed in the inferior vena cava.

The freed portion of the aorta was severed between Potts clamps and an Eckstein cannula was placed between the 2 aortic segments. During the installation of the aortic cannula in order to prevent cerebral hypertension blood was allowed to run into the reservoir attached to the left carotid artery and as soon as the Potts clamps were released the blood was pumped back into the animal. The Eckstein cannula directed the blood through the Shipley rotameter and back to the aorta. A side branch of this cannula was introduced into the right innominate artery to maintain the blood circulation to the brain as both innominate vessels were ligated thus shunting all the aortic blood flow (exclusive of coronary circulation) to the rotameter (Figure 1).

*From the Department of Surgery, Western Reserve University School of Medicine at City Hospital of Cleveland, Ohio. This work supported in part by Grant Number H 1317 (C3) from the National Institute of Health.

left side of the heart. Under these circumstances, if the pulmonary ventilation is constant the $p\text{CO}_2$ of arterial blood will rise and the oxygen saturation will fall. This rise and fall will be roughly proportional to the amount of lung collapsed. Hyperventilation prevents the rise in $p\text{CO}_2$ of arterial blood but does not prevent a fall in oxygen saturation. The hyperventilation washes out a more than normal amount of CO_2 in the blood passing through functioning alveoli, thus compensating for the unaltered amount of CO_2 in the shunted venous blood. The hyperventilation however does not result in a significant increase in the amount of oxygen held by the blood passing through the functioning alveoli. The hemoglobin in this blood is already completely saturated, or almost so, and the amount of oxygen in physical solution (normally 0.15 ml/100 ml of blood) will not be significantly increased by the hyperventilation. From a clinical standpoint the surgeon should be aware that while part or all of the lung is collapsed hypercapnia can be avoided by hyperventilation but that hyperventilation will not relieve the associated hypoxemia. Periodic re-expansion of the lung is probably advisable to provide intermittent normal saturation of the arterial blood with oxygen.

SUMMARY

1 In 6 patients the total pulmonary ventilation, carbon dioxide tension and per cent saturation of arterial blood with oxygen were determined during steady states in the course of operations involving an open thoracotomy.

2 No increase in pulmonary ventilation was required to maintain the carbon dioxide tension of arterial blood at a constant level with the chest open and the lung expanded.

3 Compression of the lung with one exception required an increase in pulmonary ventilation to maintain the carbon dioxide tension of arterial blood at the same level.

4 With the lung compressed even with hyperventilation in most cases the per cent saturation of arterial blood with oxygen fell.

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on the mean systemic arterial blood pressure consisted of a progressive rise to about 125 per cent of the control reached in about 1 min after the induction of hypoxia. This rise was momentary and was followed by a gradual and progressive drop that reached the zero level in about 6 min.

The pressure in the left auricle and in the pulmonary artery progressively rose up to a peak that was reached in about 2 min. This was followed about 1 min later by a progressive rise in the venous pressure. This rise maintained itself at the same level till the end of the experiment.

The heart rate steadily fell from the start of the experiment and reached its lowest level in about 3 min. At this point the heart became dilated and it came to a standstill in about 6 min despite the presence of a recordable electrocardiographic activity several minutes thereafter.

As illustrated in graph 2 Figure 3 the aortic blood flow steadily and progressively decreased until it stopped in about 5 minutes.

In contradistinction to the decrease in the blood flow the total systemic arterial peripheral resistance calculated after the formula of Fowler *et al*⁴ increased markedly until circulation of blood ceased.

DISCUSSION

The progressive decrease in the systemic arterial blood pressure after the initial rise associated with a concomitant decrease in the blood flow speaks in favor of the inefficiency of the left ventricle in maintaining its role as a mechanical pump. The increase in the venous pressure is indicative of right ventricular failure.

Study of the time relationship between the failure of the left and right ventricles reveals that the pressure in the left auricle rises about 1 min after the systemic arterial blood pressure starts to drop; the rise in the pulmonary artery pressure comes about 20 sec after the rise in left auricular pressure. The venous pressure increases about 1 min after the rise in the pulmonary

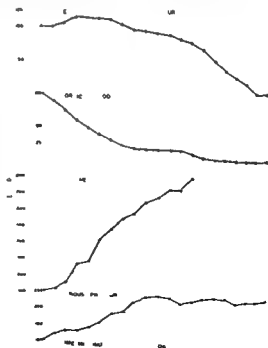


Fig 3 Time relationship between the mean arterial blood pressure, aortic blood flow, peripheral resistance and venous pressure expressed in percentage of the control values obtained before the induction of hypoxia.

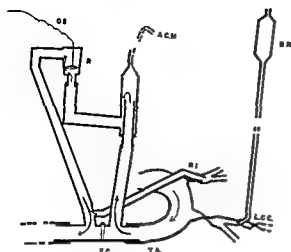


Fig 1 Illustration of Experimental Set up OS is Ocillograph R Rotameter ACM is Air Chamber Dumper BR Blood Reservoir RI Right Innominate Artery LCC Left Common Carotid EC Eckstein Cannula TA Thoracic Aorta

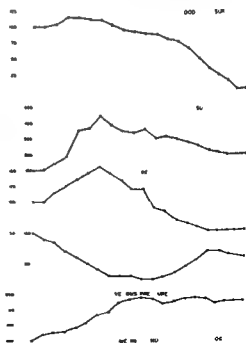
Control readings were made after equilibrium had been established for a period of 10 min. The animal was then made hypoxic by the administration of a respiratory mixture containing 99.5 per cent nitrogen and 0.5 per cent oxygen through the intratracheal tube. Pulse electroencephalogram, electrocardiogram, aortic blood flow and pressures in left auricle, inferior vena cava and femoral and pulmonary arteries were continuously recorded. Arterial blood samples were drawn at intervals of 2 to 3 min. for analysis.

RESULTS

Under the experimental conditions the arterial blood oxygen content progressively fell to 0.5 volume per cent within 5 min. after the start of nitrogen administration. The arterial $p\text{CO}_2$ remained near normal until circulation ceased.

As shown in graph 1 (Figure 2) the effect of this acute and severe hypoxia

Fig 2 Time relationship between the mean arterial blood pressure, pressure in left auricle and pulmonary artery, heart rate and venous pressure expressed in percentage of the control values obtained before the induction of hypoxia.



DYNAMICS OF PLEURAL EFFUSIONS*

ROY H. CLAUS, HAOI YACOBIAN AND HAROLD G. BARKER

The hydrodynamic factors governing the accumulation, maintenance and resolution of pleural effusions are largely unknown. The underlying diseases which lead to such collections and the usual clinical course are better known but the basic physiology may seem dissimilar in varying clinical conditions. It is apparent that if differing hydrodynamic patterns could be found to characterize the various states, an approach would be made to a better understanding of the physiology. With this in mind we have studied 20 patients who had pleural effusions secondary to tuberculosis, carcinoma, non-tuberculous infection and spontaneous pneumothorax. To date some groups are too small to permit conclusions but as yet no characteristics have emerged which distinguish the hydrodynamics of the various groups.

METHOD

Deuterium oxide was used as a tracer for water in the closed 2 compartment system where 1 compartment is the pleural space and the other is the remainder of the total body water. In some cases the tracer was injected intrapleurally and in others it was injected intravenously. In either case a figure for the volume of the sum of the two compartments (total body water) is easily calculated so long as equilibrium is reached. Where the tracer is injected intrapleurally, a figure for the volume of the primary compartment (pleural fluid) can easily be calculated by extrapolation of the semilog plot of the concentrations of the tracer in pleural fluid samples. Where the primary compartment is all the body water except the pleural fluid (intravenous injection) the calculation of the volume of the primary compartment by extrapolation becomes inaccurate due to the slow rate of change in concentrations. We found it preferable in these cases therefore to determine the pleural fluid volume instead using an independent tracer (T 1824 dye). Likewise it was deemed advisable to base the measurement of half life on the slope of the semilog plot of concentrations of the tracer in pleural fluid whether the pleural fluid represented the primary compartment (compartment of injection) or the secondary compartment (compartment of diffusion).

The early experiments demonstrated that equilibrium between the compartments is usually achieved within 12 hr, occasionally earlier but some times not until about 24 hr. Patients who had normal cardiodynamics were selected and they were studied under conditions of normal hydration. Using local anesthesia polyvinyl tubing was introduced into the pleural space through a thin walled 14 gauge needle and the needle then withdrawn. A needle adapter and three way stopcock were attached and 20 mg. of heparin injected to prevent coagulation. After drawing a control sample of small volume a volumetrically measured quantity of Evans blue dye was injected intrapleurally followed by an accurately weighed dose of heavy water (up

*From the Department of Surgery, Columbia University College of Physicians and Surgeons and the Surgical Services of the Bellevue Hospital (Chest Service) and the Presbyterian Hospital. This study supported in part by U. S. Public Health Grants G 4230 and RG 2926 (C-4).

artery pressure. In other words, the left ventricle fails first and, subsequently back pressure causes the right ventricle to fail.

The initial rise in the systemic blood pressure obtained in this experiment is comparable to the results of Gellhorn⁵ who used 85 per cent oxygen and nitrogen mixture in the human and to those of Clowes *et al*¹ who used 0.5 to 1 per cent oxygen and nitrogen mixture in the dog.

The left ventricular failure starts by bradycardia as illustrated in graph 2. Subsequently the bradycardia leads to a mechanical standstill of the heart. This occurs despite recordable electrocardiographic activity. These results support the teaching that the common myocardium (the contractile portion of the heart) will not function in the absence of oxygen while the specific tissue (sinus node, auriculoventricular node and bundle of His) will function rhythmically without oxygen.⁸

The total systemic arterial resistance rose throughout the experiment as long as blood flow continued. This means that in acute and severe hypoxia the peripheral vasoconstrictor mechanisms do not fail but display overactivity as evidenced by the increase of the peripheral resistance and the forwarding of adequate venous return leading to an increased venous pressure.

SUMMARY AND CONCLUSION

From the data obtained from dogs subjected to acute severe hypoxia it is concluded that the primary cause of circulatory arrest is failure of the heart to perform its work rather than a fall in arterial resistance or venous tone. The mechanical failure of the heart occurs despite recordable electrocardiographic activity.

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formulae used above have been derived by others (in slightly different form) who have written on the closed two compartment system^{1,2,3}

RESULTS AND DISCUSSION

The results are presented in Table 1. We encountered occasional patients in whom equilibrium was prolonged beyond 21 hr. or was never achieved at all. Such patients were found to retain D₂O and/or Evans blue in their pleural space at unreasonably high concentrations and it could only be assumed that their effusions were loculated thus preventing the collection of representative samples. These subjects have been eliminated from the report. In the 2 subjects with spontaneous pneumothorax an artificial pleural effusion was created by the injection of isotonic saline solution to see if the turnover rate might be different from that in patients with spontaneous effusions. It is seen that the rate is of the same general order of magnitude. It should be noted that there is considerable individual variation in the exchange rates and this does not seem to depend upon the size of the effusion or upon the disease process involved although too few studies have been done in non tuberculous effusions to be sure about this.

The high rates of turnover found (30 to 75 per cent per hour) are of interest since they are similar to the high rates of turnover described for ascitic fluid.² In a few patients we have also studied the turnover rate of sodium in pleural fluid using Na²⁴ as a tracer and have found that the turnover rate is somewhat slower than that of water. Total body water in some of our subjects was found to be higher percentagewise than one might expect but this was felt to be due to loss of body fat with their disease rather than additional body water accumulation over and above the pleural fluid.

SUMMARY

Rate of pleural fluid turnover has been measured by a tracer technique in a group of patients with pleural effusions from several different disease processes. We have not been able to detect a difference in exchange rate charac-

Table 1 Pleural Fluid Exchange Rates

PT	AGE	SEX	VOL (L)	PLEURAL FLUID	
				L/HR.	EXCHANGE RATE %/HR.
W.S.	61	M	10	30	30
C.K.	45	M	12	49	41
R.C.	29	M	24	72	30
W.L.	58	M	18	104	38
M.V.	24	F	6	19	32
J.C.	33	M	24	72	30
W.M.	21	M	14	61	44
M.F.	20	F	15	74	49
F.J.	39	F	27	145	54
J.L.	32	M	13	69	53
S.M.	37	M	18	71	39
F.T.	29	M	11	83	75
H.B.	37	M	12	68	57

E.J. had carcinoma. J.L. Friedlander type B pneumonia. S.M. constrictive pericarditis. F.T. and H.B. artificial effusions, the remainder all had tuberculous effusions.

proximately 50 gm) either intrapleurally or intravenously. Mixing of the contents of the pleural effusion was then accomplished by a standard exercise of two deep breaths in each lateral position upright and prone. The patients then rested supine. A multiple syringe technique was employed for removal of specimens and the volumes of pleural fluid removed were as small as possible (no more than 3 or 4 cc each). Specimens were withdrawn at 20 to 30 min intervals for 4 samples and then at longer intervals up to 24 hr. Simultaneous pleural fluid and blood samples were drawn throughout in some subjects and in others bloods were drawn only near the conclusion to demonstrate equilibrium. A pretest overnight fast was continued until after the first 4 specimens had been obtained after which fluids and foods were given at a rate approximating loss. No fluids were permitted in the 2 hr preceding any sampling. Evans blue determinations were carried out in a Beckman model DU spectrophotometer. Heavy water was determined by the standard falling drop method following distillation of pleural fluid and serum samples. Calculation of pleural fluid volume from Evans blue dye was based on straight dilution since its concentration did not decline appreciably with time but several samples were always drawn and analyses averaged. Total body water was calculated from the D_2O figures by the usual method converting weight of dose given into volume at body temperature using the figure for percent purity of the standard and known data for specific gravity of heavy water at body temperature. A very small correction was made for assumed urinary and insensible loss of dose in the calculation of total body water since this calculation was based on the 12 or 24 hr equilibrium samples. No similar correction was made however in the earlier figures which went into the determination of the half life slope and intercept on the semilog plot.

For the calculation of exchange rate of pleural fluid the individual concentrations of tracer in pleural fluid were subtracted from the equilibrium figure (in the case of intravenous administration) and the resulting data plotted on semilog paper against time. When D_2O was injected intrapleurally the subtraction was reversed. The slopes and intercept of the resulting lines were then read in the latter case and only the slope in the former. Calculations were based on the following formulae:

$$(1) P_t = (P_0 - P_{eq})e^{-(k_p + K)t} + P_{eq}$$

$$(2) S_t = -S_{eq}e^{-(k_p + K)t} + S_{eq}$$

$$(3) P_t - S_t = P_0e^{-(k_p + K)t}$$

where P = concentration in primary compartment

S = secondary

t and eq are times

k_p is fraction of the volume of primary compartment exchanged per unit time

K is the same for the secondary compartment

It is obvious from Equation 2 that the intercept of the straight line will be at the equilibrium concentration thus making it impossible to calculate the volume of the secondary compartment based on data in this compartment alone and necessitating the use of the independent tracer Evans blue. The

METHOD

The veins used were taken from dogs under strictly sterile techniques. Each vein was placed in a glass tube previously sterilized by autoclave, which contained 2 cc of Ringer's solution, 500,000 u of penicillin and 1/2 gm of dihydro-streptomycin. The tubes containing the veins were immediately placed in the freezer where they were kept at 8 to 10°C below zero. From the time that the vein was extracted for preservation, small fragments were periodically subjected to careful histologic study and bacteriological control. Before being taken to the operating room for use, the fragments of vein were subjected to a final histologic and bacteriological study (Fig 1).

During the course of this experiment 200 dogs varying in weight from 8 to 25 kg and in age from 1 to 7 yr were anesthetized. Ether or nembutal was used as anesthetic, the latter dosage being from 1 to 6 cc/kg of weight given intraperitoneally and using in both cases intratracheal oxygen positive pressure with our own design semi-automatic anesthetic machine for intrathoracic work in animals.

The left hemithorax was then opened by subperiosteal resection of the 5th rib. A left pneumonectomy was then performed using the usual technique. In 100 dogs the left bronchus was closed by separated stitches of #00 Pagenstecher linen. The fragments of superior vena cava had been carefully thawed first by Balneum Marie and then by the application of warm compresses. The open fragment was then placed over the bronchial stump in the form of a cap covering it well and then sutured to the external bronchial wall by separate stitches of Deknatel #5/0. We have also used continuous stitching with the same material and obtained very satisfactory results.

After we made sure that the vena cava completely covered the bronchial stump and that it was correctly sutured, positive pressure was augmented considerably to assure that the technique had been correctly applied and that there was no escape of air even under exaggeratedly high pressure.

The thoracic wall was closed in the usual manner. The dogs received postoperative penicillin intramuscularly 100,000 u each 12 hr during the first 6 days.

The other 100 dogs were used as a control group.

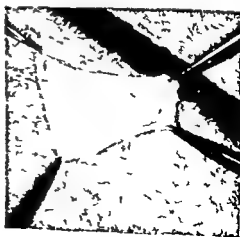


Fig 1 Vena cava graft opened and extended ready for use

terizing different diseases. Likewise the rate in 2 patients with normal pleurae was measured after creation of artificial effusions by the injection of saline solution and found to be similar to the rate in patients with spontaneous effusions.

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THE USE OF FROZEN VENA CAVA GRAFTS TO AID THE CLOSURE OF THE BRONCHIAL STUMP FOLLOWING PNEUMONECTOMY*

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LIMA AND SALVADOR DIAZ GUTIERREZ

One of the problems that still vexes resectional pulmonary surgery is the appearance of bronchial fistulas especially as the aftermath of extirpation of the superior lobes and pneumonectomies in tuberculous patients. Diverse methods of covering the sutured bronchi and even special techniques for suturing have been reported.

As has been observed by various authors in a large number of patients the percentage of success is high whether the method used be that of re-covering the bronchi with peri bronchial tissue or with parietal pleura, pericardium, fatty tissue, etc. We must accept the fact, however, that a number of patients develop total or partial ruptures of the bronchi after resection.

The object of this paper is to report the following experimental study in which dogs subjected to pneumonectomy were treated by covering the stump of the sutured bronchi with pieces of the superior vena cava which has been preserved by freezing. This study covers a period of 3 years and was done at the Department of Surgical Investigation of the University of Guadalajara, Mexico.

The reasons that persuaded us to decide upon the use of the vena cava to cover the sutured bronchi were the histologic manifestations of fibrous reaction and organization observed in arterial and vein grafts used as vascular substitutions, and consistence of the venous tissue.

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ing two dogs presented fistulas and were sacrificed for observation. In both purulent liquid was found in the cavity. When sacrificed the general condition of these dogs was very poor.

Examination of the Stump In a gross study, the group where the stump was capped by venous grafts showed considerable firmness in the area of the stump after the third day. This aspect was progressive during the following days, and by the end of the tenth day had acquired a very consistent aspect. The control group also tended to rapid firmness, but we believe that there was greater consistency in the first group (Figs 2a, 2b, 2c).

Microscopic Study Usually after 24 hr at the point of contact of the venous cava with the bronchus there could be observed a fibrous exudation which englobed inflammatory cells. After 48 hr, inflammatory tissue with young fibroblasts and great capillary organization was observed on the external surface of the vein. As time passed the conjunctive proliferation became more intense and firm, the fibrinous exudation disappearing until there remained a true fibrous frame (Fig. 3a, 3b, 3c, 3d).

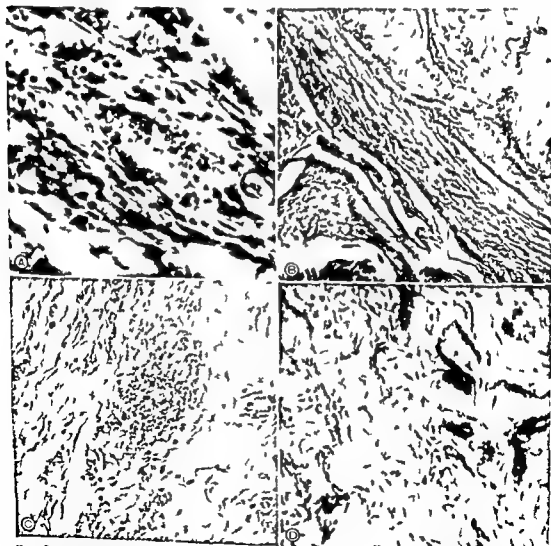


Fig. 3 (a) Young fibroblastic exudation with inflammatory cells and capillary organization 48 days after bronchial venous covering. (b) Fibrous organization after 10 days. (c) Healing process at 60th day. (d) Conjunctive sclerosis at 105 days.

Table 1 Experiments Bronchial Closure in Pneumonectomy

1—WITH AID OF VENA CAVA GRAFT		
NO OF DOGS	DEATHS	FISTULAE
100	8 8%	0 0%
2—WITHOUT VENA CAVA GRAFT		
100	12 12%	5 5%
Total 200	20 20%	5 5%

They were also subjected to left pneumonectomy, the same surgical technique being used as with the other group. However in this control group the bronchial stump was not capped with frozen vena cava grafts but was covered by peribronchial tissue.

Both groups were subjected to the same postoperative treatment. All received postoperative penicillin for 6 days. Over post surgical periods varying from 24 hr to 8 mo a number of animals was sacrificed for study.

DISCUSSION

Mortality In the group subject to pneumonectomy in which the bronchial stump was capped by frozen vena cava grafts there were 8 deaths due to pneumonitis, atelectasis and cardiac failure (8 per cent). In the control group there were 12 deaths, 7 from pneumonitis and atelectasis and 5 from empyema with bronchial fistula (12 per cent).

Morbidity In the group where the bronchial stump was capped by frozen vena cava grafts no case of bronchial fistula appeared and the formation of liquid in the cavity was noticeably reduced.

In the control group where the bronchial stump was covered with peribronchial tissue 5 bronchial fistulas occurred, 3 of which provoked empyema which we interpreted as leading to the death of these animals. The remain-

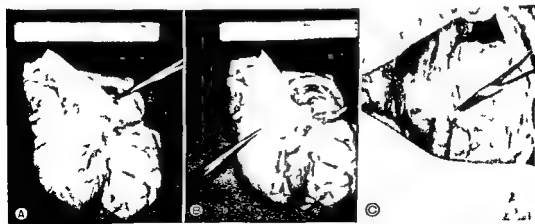


Fig 2 (a) Dog sacrificed 10 days after left pneumonectomy showing venous covering of the left main bronchus. 2 (b) Specimen with trachea and main bronchus opened longitudinally. 2 (c) Left pleural cavity 20 days after pneumonectomy. Forceps points to area of bronchial venous covering.

ing two dogs presented fistulas and were sacrificed for observation. In both purulent liquid was found in the cavity. When sacrificed the general condition of these dogs was very poor.

Examination of the Stump. In a gross study, the group where the stump was capped by venous grafts showed considerable firmness in the area of the stump after the third day. This aspect was progressive during the following days and by the end of the tenth day had acquired a very consistent aspect. The control group also tended to rapid firmness, but we believe that there was greater consistence in the first group (Figs 2a, 2b, 2c).

Microscopic Study. Usually after 24 hr at the point of contact of the venous graft with the bronchi, there could be observed a fibrous exudation which englobed inflammatory cells. After 48 hr, inflammatory tissue with young fibroblasts and great capillary organization was observed on the external surface of the vein. As time passed the conjunctive proliferation became more intense and firm, the fibrinous exudation disappearing until there remained a true fibrous frame (Fig 3a, 3b, 3c, 3d).

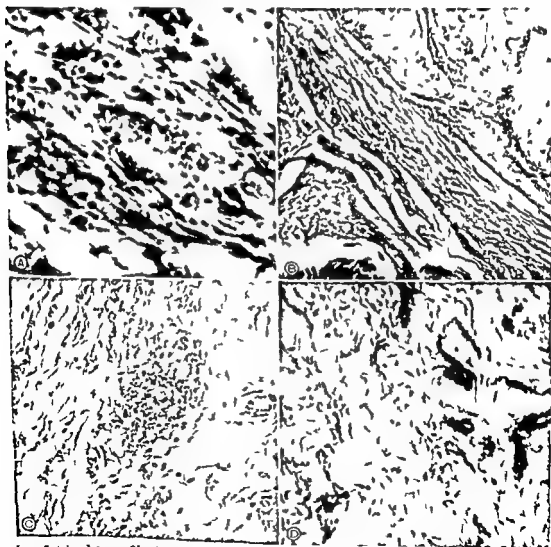


Fig. 3 (a) Young fibroblastic exudation with inflammatory cells and capillary organization 48 days after bronchial venous covering. (b) Fibrous organization after 10 days. (c) Healing process at 60th day. (d) Conjunctive sclerosis at 105 days.

The vein graft behaved as a foreign body which became encased by conjunctive proliferating tissue on the internal part of the vein but which originated on the bronchus and the external part of the same portion of the vein where after the sixth day it acquired considerable firmness

A group of 12 human patients have been treated with this technique using pieces of human superior vena cava. Postoperative control has passed the eighteenth month in one of them. Observations to date have been satisfactory and we hope in the future to be able to present the results of a conclusive number of cases

CONCLUSIONS

1 An experimental study in 200 dogs is reported. In 100 animals pneumonectomy was performed capping the sutured bronchial stump with a frozen vena cava graft preserved at 8 to 10°C below zero. The other 100 dogs were used as a control group.

2 The mortality and morbidity were considerably lower in the group where the stump was capped by the vena cava graft.

3 Histologic studies postoperatively demonstrated the existence of a good fibrous reaction which gave firmness and consistence to the bronchial stump.

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CARDIOPULMONARY RESERVE IN 10 TO 15 YEARS FOLLOWING FIFTY PER CENT OR MORE REDUCTION OF LUNG VOLUME *

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WILLIAM L. ADAMS

Extensive pulmonary resection frequently results in marked alterations of cardiopulmonary function even though pulmonary capacity under normal conditions has an enormous reserve. In certain individuals notably those in the older age group the cardiorespiratory system is unable to compensate adequately to pneumonectomy and death occurs in the early postoperative period.¹ Another group of patients undergoing extensive resection therapy is able to compensate sufficiently to the immediate effects of the sudden reduction in pulmonary parenchyma only to develop incapacitating symptoms of reduced cardiopulmonary reserve at a later time. Frequently these individuals are entirely asymptomatic at rest only to experience severe dyspnea on slight exertion. The late effects experienced by these individuals are not readily attributable to a lack of functioning lung tissue but are more likely due to a pulmonary vascular insufficiency with pulmonary arterial hypertension. The late effects of pneumonectomy will be of ever increasing importance with its widespread use today as the operation of choice for carcinoma of the lung.

A marked decrease in the amount of functioning lung in normal dogs produces alterations in the right ventricular pressures. A study from this laboratory showed that a 75 to 85 per cent reduction of vascular bed by resection of lung tissue in dogs resulted in immediate and sustained elevation of right ventricular pressure of the order of 90 per cent.^{2,3} In an attempt to better understand the mechanisms involved in the late effects of reduced lung volume a group of patients and animals that had undergone extensive resection of lung tissue 5 to 15 yr previously were studied with respect to their cardiopulmonary responses at rest and during exercise.

METHOD

Six dogs that had experienced 50 to 75 per cent reduction in functional lung volume 10 or more years previously were studied. Three of these animals had undergone staged pulmonary resections resulting in only the right upper and middle lobes remaining. The other 3 animals had their lung volume reduced by a combination of resection and the production of bronchial stenosis and resultant atelectasis.

Right ventricular or pulmonary artery catheterization was performed under local anesthesia as was cannulation of the previously transplanted subcutaneous carotid artery. Strain gauge manometers were employed for pressure determinations. A Wood cuvette with recording oximeter was attached to the cardiac catheter and carotid cannula allowing the periodic withdrawal

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of blood samples for the determination of arterial and mixed venous oxygen saturations. The dogs were exercised by requiring them to run on a tread mill at a speed of 1.75 miles per hour for periods of 2 to 5 min.

Seven patients who had undergone pneumonectomy 5 to 15 yr previously were investigated. They ranged in age from 45 to 73 yr. Each patient was evaluated clinically with regard to his present occupation and activities in an attempt to classify present exercise tolerance.

Functional capacity was graded on a scale of I to IV. Class I included those patients having no limitation of physical activity and ordinary physical activity causing no discomfort. Class II signified a slight to moderate limitation of physical activity, Class III a moderate to great limitation of physical activity while Class IV was assigned to the patients unable to carry on any physical activity without discomfort.

Right heart catheterization was performed on each patient for measurement of right ventricular or pulmonary artery pressures. Arterial oxygen saturation was determined by means of the Wood earpiece and recording oximeter.⁴ Mild to moderate exercise was accomplished by the patient bicycling against resistance while supine. The duration of the exercise period was 1 to 4 min depending upon the abilities of the patient. Observations of alterations in right heart pressure, systemic blood pressure, arterial oxygen saturation and pulse rate were made during the period of exercise.

RESULTS

Animals. The 3 animals that had undergone 75 per cent resection of lung tissue have been observed periodically with respect to right heart pressures. In all cases there was an immediate marked increase in pulmonary artery pressure over the preoperative control level of the order of 67 to 188 per cent. Over the course of 6 to 7 years the pulmonary artery pressures have gradually decreased to a level only 2 to 10 mm Hg above the preoperative levels. Nevertheless, all of the animals with markedly reduced functional lung volume of long standing when subjected to exercise showed a 36 to 84 per cent increase in pulmonary artery pressure over resting levels with an average increase of 64 per cent for the group. The long term pulmonary artery pressure studies as well as the response to exercise are represented graphically in Figure 1.

In general, the alterations in arterial oxygen saturation were less striking with an average decrease of 3 per cent. The systemic blood pressure and pulse rates showed increases of a variable degree during the exercise period.

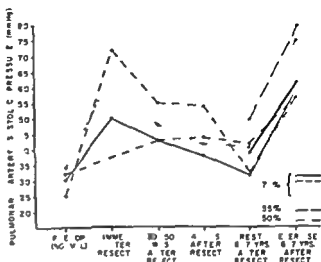
Patients. Six of the patients were working full time in relatively sedentary occupations. The other patient, J.S., complained of slight dyspnea at rest and approximately 3 mo previously had been in frank congestive heart failure. Three of the patients denied any dyspnea on ordinary activity while the other 4 described varying degrees of exertional dyspnea.

Two patients had resting pulmonary artery pressures within the range of normal. The resting pulmonary artery pressures in 3 others were slightly elevated (25 per cent) while in the remaining two patients the pulmonary artery pressures were increased two and threefold over the normal value at rest.

In every patient the pulmonary artery pressure rose with the beginning of exercise and was sustained throughout the exercise period. With the ces-

PULMONARY ARTERY SYSTOLIC PRESSURES IN DOGS
FOLLOWING 50-75 PERCENT REDUCTION IN LUNG VOLUME

Fig 1 Pulmonary artery systolic pressures in dogs following a 50 to 75% reduction in functional lung volume. All pressures except the last column were measured in awake resting animals. The "exercise" pressures were measured while the animals were running on a treadmill at 17.5 mi/hr. The heavy dark line connecting the last 2 columns illustrates the average pulmonary artery pressure response of the 6 animals.



sation of exercise the right heart pressure showed a gradual fall but usually did not return to pre-exercise levels within 10 to 15 min. The rise in pulmonary artery pressures was 15 to 49 mm Hg representing 45 to 140 per cent increases over the resting levels. The average increase in right heart pressure for the group of patients was 29 mm Hg representing a 72 per cent increase over the average resting value of 40 mm Hg.

The resting arterial oxygen saturation was normal to only slightly depressed. The effect of exercise was in general less striking than in the case of the pulmonary artery pressures but in general (6 of 7 cases) the saturation decreased during the exercise period. This decrease ranged from 2 to 14 per cent with an average of 5 per cent for the group. The individual alterations in pulmonary artery systolic pressure and arterial oxygen saturation are summarized graphically in Figure 2. The systemic blood pressure and pulse rate increased during the exercise period as would be anticipated.

DISCUSSION

The pulmonary hypertension developing in animals as a result of marked reduction in lung volume gradually tends to return toward normal levels over the course of 6 to 7 years. These compensatory changes in the pulmonary vascular bed, however, are not of such a nature or degree as to allow a normal response to exercise, i.e., slight to no rise in pulmonary artery pressure.

The patients studied revealed a fairly close correlation between their functional classification and the level of pulmonary artery pressure at rest and its alteration during moderate exercise. No correlation was apparent between the patient's age or duration of reduced lung volume and his functional class or pulmonary artery pressures. However, it is our impression that pulmonary hypertension is more significant in the older age group of pneumonectomy patients.

The normal cardiopulmonary response to exercise is one directed toward increasing the amount of oxygen uptake in the lungs to fulfill the increasing oxygen demand of the body tissues. The oxygen uptake in the

PULMONARY ARTERY PRESSURES AND ARTERIAL OXYGEN SATURATIONS IN PATIENTS 5 TO 15 YEARS FOLLOWING PNEUMONECTOMY

R I C D E S S I O P

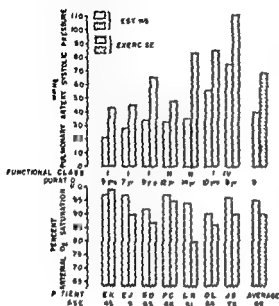


Fig 2 Comparison of the effect of exercise on pulmonary artery systolic pressures and arterial oxygen saturations in post pneumonectomy patients (arranged according to functional class — see text). Exercise consisted of moving the legs against resistance in the supine position.

lungs may be increased either through increases in cardiac output or in arterial venous oxygen differences. In normal individuals during light exercise the increase in cardiac output greatly predominates and the change in arterial venous oxygen difference is minimized. Exercise in the normal individual may increase cardiac output 2 to 2½ times with a slight elevation of systemic blood pressure while the right heart pressure and arterial oxygen saturation remain essentially unaltered.⁴

The pulmonary artery pressure represents a summation of the left atrial pressure and the pressure gradient between the right ventricle and left atrium. This gradient, by Poiseuille's law, is directly proportional to cardiac output and inversely proportional to peripheral resistance, a factor dependent primarily upon the number and caliber of vessels in the pulmonary bed.

To account for the essentially unchanged right heart pressure in the normal individual during exercise with an increase in cardiac output, the pulmonary arterial resistance must be decreased by an increase in pulmonary vascular volume. Riley *et al*⁵ found that the pulmonary vascular resistance decreased to approximately one third of its resting value during severe exercise. The reduced pulmonary vascular volume in the post pneumonectomy patient apparently often requires a higher than normal pressure gradient to maintain a normal rate of blood flow. Furthermore, this reduced pulmonary vascular bed is less able to accommodate the increased blood flow accompanying exercise, thereby imparting increased vascular resistance and leading to an elevation of the right heart pressure. It is not clear whether there is a fixed and restricted pulmonary vascular capacity in this situation resulting from simple volume reduction and intimal and arterial sclerosis or if there is in addition a reflex vascular change. The latter factor has not been elucidated, yet so no therapy can be considered for it. In recognizing the former factor, the most valuable therapy is limitation

of extent of resection whenever feasible and instruction of the patient in restriction of his activities.

SUMMARY

1. Resting right heart pressures of dogs with a 50 to 75 per cent reduction in functional lung volume show an average immediate increase of 90 per cent which tends to decrease over the course of 6 to 7 years to levels near normal. However, moderate exercise of these animals produces an average increase of 61 per cent in the pulmonary artery pressure.

2. Patients 5 to 15 years post pneumonectomy have a functional capacity that correlates with the degree of pulmonary hypertension. Resting pulmonary artery systolic pressures average 10 mm Hg and increase an average of 72 per cent during moderate exercise. Exercise usually results in a decrease in peripheral arterial oxygen saturation.

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A STUDY OF THE BRONCHIAL ARTERY FLOW IN THE DOG*

DAVID STATE PETER F SALISBURY AND PETER WEIL

Anatomical studies have demonstrated dilatation of the bronchial vessels in congenital heart disease and in acquired pulmonary lesions^{1, 2, 3} The observed morphological changes parallel alterations of bronchial flow in certain congenital cardiac abnormalities the bronchial arteries may carry up to 50 per cent of the cardiac output² The flow in isolated bronchial arteries has been measured with flow meters,⁴ and there have been attempts to measure the total bronchial flow^{5, 6} in isolated preparations The development of effective and manageable equipment for total body perfusion has afforded us the opportunity to measure bronchial flow by a technique utilizing separate perfusions of the greater and lesser circulation

METHOD

The acute experiments described here were performed on 14 mongrel dogs 8 to 30 kg in weight They were anesthetized with pentobarbital sodium, 5 mg/kg and given heparin 2 mg/kg intravenously Pressures in the femoral artery and in a branch of the pulmonary artery were registered with plastic cannulae connected to strain gauges amplifiers and a multi channel direct writing oscillograph The heart and great vessels were exposed under positive pressure endotracheal respiration by a bilateral trans sternal thoracotomy through both 4th intercostal spaces A plastic tube (Bardic No 21 French) for the withdrawal of venous blood was inserted into the right ventricle through the right auricular appendage When a ligature about the pulmonary artery was tied all venous blood from the right heart was then diverted through the heart lung machine All blood from the left ventricle was drained into a graduate reservoir which was placed 10 cm below the dog's spine Blood from this reservoir was re-injected by a variable speed Sigmamotor pump into the pulmonary artery With this arrangement blood

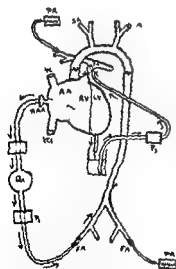


Fig 1 System for independent perfusion of the systemic and pulmonary circulation R1A right auricular appendage R1 right atrium R1 right ventricle L1 left ventricle ACS vena cava superior VCI vena cava inferior Ao aorta P1 pulmonary artery S1 subclavian artery C1 carotid artery FA femoral artery P1 pumps O₂ oxygenator RFS reservoir PR pressure recording Arrows indicate direction of blood flow Changes in reservoir level indicate changes in bronchial artery flow

*From the Department of Surgery and the Institute for Medical Research Cedars of Lebanon Hospital Los Angeles California Supported by a grant from the Los Angeles County Tuberculosis and Health Association

which issued from the pulmonary veins could only come from the bronchial arteries or from blood pumped into the pulmonary artery. Since the latter drains into the reservoir, any rise in blood level in this collecting chamber must be due to bronchial flow. The quantity of this flow per standard observation period can thus be read directly from the blood level in the reservoir.

The systemic circulation of the animals was perfused with arterial flows ranging from 25 cc/kg/min to 70 cc/kg/min. This resulted in systemic arterial pressures ranging from 40 to 110 mm Hg systolic. Pressures in excess of this were observed after administration of phenylephrine hydrochloride (neosynephrine (R)). Blood was perfused into the arterial tree via the femoral or subclavian artery with a distinct pulsatile quality. Back leakage of the blood through the aortic valve was prevented when necessary by a Blalock clamp on the ascending aorta.

A short period of perfusion of both circulations was necessary to obtain a steady state before observations were made.

Effect of Variation of Systemic Arterial Pressure Upon Bronchial Artery Flow. The immediate increase and decrease of bronchial flow with corresponding changes of the systemic pressure was one of the most consistent and reproducible findings in this series of experiments. When the systemic arterial pressure is kept constant (with small amount of hexamethonium) while the arterial perfusion is increased, increased flow in the peripheral arteries did not cause a corresponding increase of flow in the bronchial vessels; this indicates that the systemic pressure and not the systemic flow is the effective agent which influences bronchial flow. At systolic arterial pressures of 90 to 100 mm Hg the average flow in the bronchial arteries was equal to 0.5 to 1.0 per cent of the total arterial blood flow.

Effect of Variation of Pulmonary Artery Perfusion upon Bronchial Artery Flow. When other factors were kept constant but the flow in the pulmonary circuit was varied, consistently reproducible changes were not observed. In the main, bronchial flow remained constant regardless of the increase, decrease or even complete arrest of the pulmonary perfusion as long as the pulmonary flow did not exceed 50 ml/kg/min. When the pulmonary perfusion was arrested, the partition of bronchial flow between the pulmonary artery and the left atrium was variable and a consistent pattern was not observed.

Table 1. Effect of Variation of Systemic Arterial Pressure on Bronchial Artery Flow

DOG NO	WEIGHT IN KG	SYSTOLIC FEMORAL ARTERY PRESSURE		RISE OR FALL EFFECTED BY	BRONCHIAL FLOW IN ML/MIN	
		FROM	TO		FROM	TO
261	20	40	55	Flow through pump	2	5
		55	30		6	2
		30	50		2	5
"		50	65		5	10
		65	35		10	4
266	24	70	160	Neosynephrine	8	27
"				After		
		160	140	neossynephrine	22	8
"		140	170	Neosynephrine	8	15

Table 2 Effect of Variations of Pulmonary Artery Flow upon Bronchial Artery Flow

Dog	Weight in kg	PULMONARY ARTERY FLOW IN ml /min		BRONCHIAL FLOW IN ml /min		PULMONARY ARTERY PRESSURE		SYSTOLIC FEMORAL ARTERY PRESSURE
		FROM	TO	FROM	TO	FROM	TO	
201	20		220	5	5	0	6	55
		220	550	5	5	6	20	55
		550		5	6	20	0	55
			220	6	0	0	9	55
		220	100	2	2	9	16	30
		100		2	2	16	0	30
			220	10	10	10	11	65

Effect of Respiratory Volumes on Bronchial Artery Flow In 5 experiments deflation of the lungs and arrest of positive pressure respiration caused a decrease of bronchial flow of up to 60 per cent of the control value. In 1 additional experiment no changes were observed. Upon resumption of ventilation the bronchial flow increased in 4 out of 5 observations. In these experiments the systemic arterial pressure did not change significantly.

Effect of Hypercapnia and Acidosis on Bronchial Flow In 7 experiments total body perfusion was maintained but the ventilation of the independently perfused lungs was temporarily effected by 10 per cent CO_2 and 90 per cent oxygen. The pulmonary perfusion blood was routed through a loop which contained glass electrodes connected to a continuously registering pH meter. When 10 per cent CO_2 in oxygen was used, a rapid fall of pH of the blood in the pulmonary circuit occurred. In 5 out of 6 experiments when the systemic arterial pressure remained constant a change of inhalation gases from 100 per cent oxygen to 10 per cent CO_2 and 90 per cent oxygen resulted in an increase of bronchial artery flow up to 160 per cent above control values.

Effect of Serotonin (5 hydroxytryptamine) on Bronchial Flow Addition of 10 mg of serotonin creatine sulfate to the blood in the oxygenator caused a sustained pronounced increase in bronchial artery flow averaging 160 per cent above controlled values in 5 experiments. After serotonin the bronchial flow was elevated for periods of up to 60 min and the increased flow diminished only gradually. Repeated administration of serotonin caused further increases of bronchial flow. After the administration of serotonin the well known pronounced rise in pulmonary artery pressure was observed but systemic arterial pressure remained the same or dropped slightly.

DISCUSSION

The dual circulation in the lungs has been investigated extensively with anatomical methods. The results of such studies have suggested a potential role of the bronchial vascular system in the pathogenesis and the pathological physiology of cardio pulmonary disease. There have been several indirect and partial measurements of flow in the bronchial artery territory but the only attempt to measure total bronchial artery flow directly was performed in 1932 by Berry and Daly who perfused the pulmonary and the bronchial circuits of isolated lungs, lung thorax and lung esophagus preparations. As far as we know the total collateral blood flow to the lungs has

not previously been measured in animals with intact reflexes

When the lesser circulation is perfused separately from the systemic circulation any increase of the blood volume in the lesser circulation must come from the bronchial arteries. The term bronchial artery flow is used in this article applies only to that portion of bronchial artery flow which drains into the pulmonary veins. It is known that some collaterals exist between bronchial and azygos veins as well as the superior vena cava.⁸ The amount that flows in these channels is not known but is felt to be insignificant unless chronic obstructions in the pulmonary veins occur.

SUMMARY

1. A method has been developed for measuring bronchial arterial flow by a technique utilizing independent perfusions of the systemic and pulmonary circulation.

2. The bronchial artery flow is directly related to systemic arterial pressure. With normal blood pressures the anastomotic channels between bronchial arteries and pulmonary vein carry 0.5 to 1.0 per cent of the total circulating blood volume per minute.

3. Deflation of the lungs causes a decrease in bronchial arterial flow ranging up to 60 per cent of control values.

4. The use of 10 per cent CO₂ in oxygen to ventilate the lung (but not the pump oxygenator) increases bronchial flow markedly over control values when 100 per cent oxygen is used.

5. Changes in pulmonary artery flow rate did not consistently affect the flow in the bronchial arteries when $\dot{p}a$ flow was less than 30 ml/kg/min.

6. Administration of serotonin (5-hydroxytryptamine) markedly increased bronchial flow.

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HUMAN MEASUREMENTS INVOLVED IN TRACHEOBRONCHIAL RESECTION RECONSTRUCTION AND PROSTHETIC REPLACEMENT A PRELIMINARY REPORT ON IVAI ON SPONGE*

JOHN E. JESSEPH JOHN K. STEVENSON HARRY N. HARKINS AND
K. ALVIN MERENDINO

One facet of our approach to the general problem of tracheobronchial resection reconstruction and prosthetic replacement has dealt with a considerable body of anatomic data from the study of a group of fresh human cadavers. At that time consideration was given to only a few of the more important dimensions of the trachea and main bronchi.¹ More recently, the data have been re-examined in an attempt to determine whether or not there is any more or less constant pattern of structure of the tracheobronchial tree. If such were true one could then plan reconstructive and prosthetic procedures in advance.

Consideration will be given here to some of the important anatomic considerations which relate to the development and application of tracheal prosthetics.

METHOD

Anatomic Data. In the total of 136 bodies originally studied there were 56 (35 female, 21 male) in which observations had been made in sufficient detail for a complete analysis. Lengths and diameters of bronchi and their orifices down to and including the tertiary (segmental) bronchial orifices were available. In studying the over-all distribution of these dimensions and in attempting to correlate them with other parameters such as sex, height, race, etc., a considerable mass of tubular data was accumulated. These findings will be published in detail elsewhere.

In the study of the 56 bodies several relationships were noted to be of fair constancy. The human male trachea has a mean luminal diameter of 2.2 cm, the female of 1.7 cm. The ranges of each are 1.8 to 2.5 cm and 1.4 to 2.0 cm, respectively. All measurements were made just above the bifurcation. If the luminal diameters of the right and left main bronchi are compared by ratio, i.e., RMB/LMB , it is found that for males the ratio is 1.21 ± 0.1 . For females the ratio is also 1.21 ± 0.2 . Further, it is found that the total cross-sectional area of the two main bronchi almost exactly equals the cross-sectional area of the trachea for both sexes. Thus it can be said that the square of the radius of the trachea is approximately equal to the sum of the squares of the radii of the main bronchi. This relationship is subject to a variation of only 7 per cent, which for purposes of surgical reconstruction is a negligible error.

Tracheobronchial Prosthetic Replacement. A number of investigators have experimentally explored several methods of prosthetic replacement of excised segments of the trachea and main bronchi.^{1, 2, 4, 9} A few applications of such artificial tubular replacements have been made in humans but with

*From the Department of Surgery, University of Washington School of Medicine, Seattle, Washington. Supported in part by funds from an Institutional Grant from the American Cancer Society.

rare exceptions no method has provided even semi permanent restoration of function^{3, 4}

Probably the greatest single problem in tracheal replacement is the method of securing union between the ends of the host trachea and the prosthesis so as to ensure union and yet avoid necrosis of the tracheal stumps. Stenosis secondary to granulation tissue ingrowth, loosening of the replacement with resultant tracheal collapse and obstruction as well as leakage with infection are all hazards to tracheal substitution. With these adversities in mind a guide to a new type of prosthesis was devised.

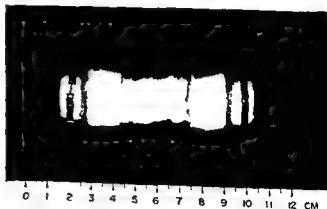
The Prosthesis. Aalon (polyvinyl alcohol) sponge has the well known property of retaining a deformity imposed and maintained during heating. Sheets of sponge can be fabricated into tubes, plugs and cylinders of any desired size and form. Compression renders the sponge less permeable and increases its strength and elasticity. Vascular grafts thus formed have shown considerable experimental promise.^{7, 10}

For tracheal replacement a cuffed tubular prosthesis is made by wrapping several thin (2 to 3 mm) layers of sponge about a smooth glass or metal rod. Narrow rings of lucite (methyl methacrylate) are placed between the layers so that the tube has radial rigidity but retains some longitudinal flexibility. At each end of the tube and likewise between the sponge layers are placed lucite rings of about 7 mm width to provide a seat for the points of the fixation ring. The sponge layers over this latter lucite ring are separated by interposing several layers of cotton tape. The final result is a cuff which can be retracted to expose each end of the prosthesis and its terminal ring (Fig. 1). Final form is achieved by wrapping, boiling and cooling.

The method of fixation of the prosthesis is a modification of Hufnagel's multiple point concept. Stainless steel bands are used, each having several sharp pointed pegs which pierce the tracheal wall and impinge upon the tubes terminal ring, preventing it from slipping out of the tracheal lumen. The ends of the fixation bands are secured by a single twist of stainless steel wire. This method does not rely on simple pressure transmitted through the wall for fixation and is consequently less likely to produce necrosis of the tracheal wall.

Experimental Application. Adult healthy mongrel dogs of either sex were used. Under Nembutal anesthesia a high right thoracotomy was performed under sterile conditions. The azygos vein was ligated and divided and the trachea circumferentially dissected free of all attachments from the anterior

Fig. 1 The Aalon sponge tracheal prosthesis. The cuffs are retracted to show the stainless steel fixation rings which hold the prosthesis in place within the trachea.



upward for a distance of about 8 to 10 cm. The trachea was then divided about 2 cm above the carina and the left main bronchus intubated so that ventilation could be maintained during placement of the prosthesis. A segment of trachea averaging 4 to 6 cm in length was excised. A prosthesis of suitable diameter was then chosen, the end cuffs retracted and one end inserted into the proximal tracheal stump. The fixation ring was then tightened about the trachea, so that its points passed through the wall and were firmly imbedded into the terminal lucite ring. The band was held in position by twisting a loop of #28 stainless steel wire passed through holes at either end. The opposite end of the prosthesis was similarly fixed into the distal tracheal stump after withdrawal of the endobronchial tube. Placing and fixing the prosthesis required only a few seconds so that there was no significant interruption of ventilation. Finally the cuffs of the prosthesis were drawn over the metal fixation rings protecting the contiguous mediastinal structures as well as providing a complete air seal (Fig. 2). Postoperatively all dogs were given antibiotics and fed a soft diet.

RESULTS

A total of 14 dogs were subjected to excision and substitution of tracheal segments as described. Of these 10 have survived and carry on all normal canine functions, the longest after an interval of 10 months. Two deaths occurred in the immediate postoperative period, 1 from a massive air leak through an unrecognized defect in the prosthesis, the other from empyema. Two late deaths (after 2 mo) resulted from the prosthesis slipping out of the trachea with subsequent intratracheal granulation tissue formation, obstruction and pneumonia. These occurred early in the series and it was found that the points of the fixation bands were too short and had not imbedded deeply enough into the prosthesis.

Each dog has been examined by serial bronchoscopy beginning in the first postoperative week. At 6 months after transplantation the trachea has a normal appearance without granulations and the prosthesis continues to fit snugly into the lumen. There is no apparent retention of secretions either in or distal to the prosthesis itself although the prosthesis reduces the lumen 2 to 4 mm—the magnitude of its wall thickness.

Bifurcated prostheses have recently been made for replacement of the distal trachea, carina and the proximal portions of both main bronchi. As yet an insufficient number of these have been placed to allow any conclusions.



Fig. 2. Operative view of an Ivilon sponge tracheal prosthesis in place with cuffs pulled over the fixation rings.

SUMMARY

Data obtained from the study of fresh human cadavers indicates that with in certain limits it is possible to predict the dimensions of certain parts of the upper tracheo bronchial tree. This is based on the finding of a considerable relative constancy of dimension among these parts.

A new tracheal prosthesis is described and some preliminary observations made following its application in dogs. A 9 mo follow up shows a high rate of survival and an excellent maintenance of respiratory and pulmonary function.

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upward for a distance of about 5 to 10 cm. The trachea was then divided about 2 cm above the carina and the left main bronchus intubated so that ventilation could be maintained during placement of the prosthesis. A segment of trachea averaging 1 to 6 cm in length was excised. A prosthesis of suitable diameter was then chosen, the end cuffs retracted, and one end inserted into the proximal tracheal stump. The fixation ring was then tightened about the trachea so that its points passed through the wall and were firmly imbedded into the terminal lucite ring. The bird was held in position by twisting a loop of #28 stainless steel wire passed through holes at either end. The opposite end of the prosthesis was similarly fixed into the distal tracheal stump after withdrawal of the endobronchial tube. Placing and fixing the prosthesis required only a few seconds so that there was no significant interruption of ventilation. Finally the cuffs of the prosthesis were drawn over the metal fixation rings protecting the contiguous mediastinal structures as well as providing a complete air seal (Fig 2). Postoperatively all dogs were given antibiotics and fed a soft diet.

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Bifurcated prostheses have recently been made for replacement of the distal trachea, carina, and the proximal portions of both main bronchi. As yet an insufficient number of these have been placed to allow any conclusions.



Fig 2 Operative view of an Ivalon sponge tracheal prosthesis in place with cuffs pulled over the fixation rings.

studies of the heart were done after perfusion of the coronary arteries with Diodrast. 1 The right ventricle was distended with fluid to determine the pressure necessary to rupture the wound. 5 Strips of myocardium cut transversely through the wound and measuring 1 cm in width were tested for disruptive strength.

RESULTS

The electrocardiographic examinations were for the most part within normal limits. In 2 dogs there was evidence of slight myocardial ischemia probably associated with the anesthesia. In none of the dogs with the myocardial thinning and fibrosis was there an abnormal tracing. In 1 dog with pulmonary artery stenosis there was right axis deviation; the others in this group were essentially normal.

In all animals some degree of adhesive pericarditis was present, especially over the scar. None had aneurysms of the ventricle nor was there a discernible alteration in heart action. In the 2 in which a clamp was utilized, scar ring through the full thickness of the myocardial wall occurred along the site of application.

Of important significance were areas of definite myocardial thinning between the wound and the anterior descending branch of the left coronary artery in 6 animals. These areas varied in size from 2.5 cm \times 1.5 cm to 0.8 \times 0.4 cm. This tissue appeared to be entirely fibrous in nature. No thrombi were present on the endocardial surface; sutures were endothelialized. No abscesses could be identified, but in 3 dogs there was extreme tissue reaction about the sutures.

Histologically, healing was characterized by dense collagen in and around the wound edges. An unexpected finding was severe chronic inflammatory response around the silk sutures in several dogs. In 1 animal with grossly discernible abnormal tissue about the sutures, the reaction was very severe even though the operation had been performed 10 months previously.

The grossly demonstrated areas of marked thinning of the ventricular wall showed almost complete absence of myocardium histologically. The wall consisted of fibrous tissue which appeared to originate from the epicardium and endocardium accompanied by considerable fat infiltration. In some areas there were a few scattered bundles of myocardium.

Visualization of the coronary arteries by the injection of Diodrast was attempted in 3 dogs and in 2 there were satisfactory films. There was no abnormality of the larger vessels. In 1 dog with satisfactory postoperative healing, the opacification of the myocardium on the side of the wound toward the left coronary artery system seemed less dense than in normal animals. This is the area in which poor healing occurred in several dogs.

In several hearts all vessels except the pulmonary artery were occluded with clamps and the right ventricle was distended with fluid. Heart valves became incompetent or the occluded vessels ruptured, but the ventricular scar remained intact although an intraventricular pressure of over 250 mm Hg was reached in 2. Continuance of this test seemed to be of little value and disruptive studies were performed on the myocardial tissue in the remaining

A method utilizing constantly increasing tension applied to the ends of a 1 cm strip of myocardium cut transversely through the scar was used to

The Heart Valvular Disease, Open Heart Surgery, Pump Oxygenator and Cardiac Arrest

STUDIES IN HEALING OF LARGE RIGHT VENTRICULOTOMIES*

WILLIAM H. MULLER JR., W. DEAN WARREN, J. FRANCIS DAMMANN JR.
AND WILLIAM H. MARSH

Further developments and refinements of extracorporeal heart lung systems have made possible the correction of intracardiac defects which heretofore were not amenable to corrective surgery. The healing of auricular wounds has presented no problem. However, the high intraventricular pressure and the fact that the ventricular wall is chiefly muscle make the ultimate fate of large ventriculotomies a potential problem.¹ It seemed timely therefore to investigate by various techniques certain aspects of healing of large ventricular wounds.

METHOD

Twenty mongrel dogs weighing 11 to 18 kg. were used. Intravenous nembutal (12 mg./lb.) was employed for anesthesia. In 2 experiments the left pleural cavity was entered through the fourth intercostal space. The heart was exposed through a large incision in the pericardium and a traction suture was placed in the midportion of the right ventricle. By placing traction on this suture a large portion of the right ventricle wall was grasped with a curved non-crushing vascular clamp and a longitudinal incision 3 to 4 cm. long was made through the full thickness. The edges were then approximated with a continuous 30 atrumatic silk suture placed 3 mm. apart through the full thickness 5 mm. from the cut edge. The pericardium was reapproximated with interrupted silk sutures and the chest was closed in the routine fashion employing water seal drainage. Subsequently 18 animals were perfused with a heart lung system and right ventriculotomies measuring 5 to 6 cm. in length were made. The myocardial wounds were closed in the manner previously described. In 5 animals pulmonary artery stenosis was created after the ventriculotomy was made to increase the right ventricular pressure and place the wound under greater stress.² Penicillin 600,000 units was given at the conclusion of the procedure. The dogs were sacrificed at intervals ranging from 2 to 16 mo.

The following studies were performed: 1. Electrocardiographic tracings were obtained before and at intervals after the ventriculotomies. 2. Immediately prior to sacrifice a thoracotomy was performed and the heart action was grossly observed. In those animals having artificially created pulmonary artery stenosis pressures were measured in the right ventricle and pulmonary artery. Gross and histologic examinations of the healed scar and surrounding myocardium were made of autopsy specimens. 3. Roentgenologic

*From the Department of Surgery, University School of Medicine, Charlottesville, Virginia. Supported by U. S. Public Health Grant #2038 for Studies on Problems Relating to the Surgical Treatment of Acquired and Congenital Heart Disease.

CONCLUSIONS

This study demonstrated no complications associated with the myocardial thinning which occurred in 6 or 30 per cent of the animals. Although these data cannot be translated directly to cardiac wound healing in patients, the defects in the myocardium must be considered a potential source of future difficulty. The problem of the healing of ventriculotomy wounds warrants further investigation.

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THE USE OF ALCOHOL IN THE TREATMENT OF THE POSTVALVULOTOMY HYPONATREMIC SYNDROME*

GEORGE J. D'ANGELO, H. V. MURDAUGH, JR., WILL C. SEALY
AND IAN W. BROWN, JR.

One of the complications following mitral commissurotomy is the syndrome of oliguria, water retention, hyponatremia and hypochloremia as described by Wilson *et al*^{1,2} and Goodyer and Glenn.³ This syndrome, although frequently transient, can become a problem in the postoperative management of seriously ill patients. Goodyer³ suggested the possibility of an antidiuretic stimulus with the hyponatremia resulting from hemodilution as in postoperative oliguria.

Leaf and Mamby⁴ reported an abnormal response to water loading in dogs made hyponatremic by dialysis. In normal dogs measurable antidiuretic activity of the plasma disappeared with water loading and diuresis occurred. In the hyponatremic dogs antidiuretic activity of the plasma was still detected following water loading and diuresis did not occur even though hemodilution was produced.

In the study of patients with the postvalvulotomy hyponatremic syndrome it was noted that they had water retention with concentrated urine of low volume in the face of electrolyte dilution as evidenced by the low serum sodium and decreased plasma osmolality. The similarity of this condition and that produced by excessive doses of pitressin together with the observations made by Leaf⁴ led to the inquiry of the possible role of the antidiuretic hormone in hyponatremic states. Since alcohol reportedly inhibits the

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determine the disruptive strength. Duplicate strips were taken from each specimen and an average was obtained. In no instance did the healed wound disrupt. In every instance including 5 hearts with gross thinning adjacent to the wound, the site of disruption was through normal myocardium or at a point of attachment of the myocardial strips in spite of special precautions in attaching the strips to the tension sources.

In the disruptive strength studies an average tension of 1650 gm. could be tolerated before disruption occurred. In the 12 hearts tested this ranged from 2100 gm. to 900 gm.



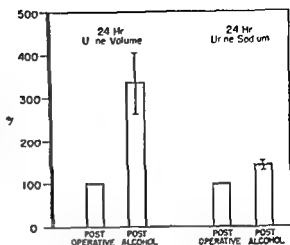
Fig. 1 A well healed ventriculotomy wound viewed through the opened right ventricle. Note the normal myocardium on either side of the incision.

Fig. 2 Healed ventriculotomy wound created by the clamp method. The inner well healed scar is the incision and the broad lateral scars are from the blades of the clamps.



Fig. 3 Ventriculotomy specimen in which a rather large area of thinning and fibrosis has occurred between the incision and the left coronary artery system. Much of this area was found devoid of myocardial tissue on histologic examination.

Fig 2 Diagram comparing the change in urine volume and urinary sodium output following alcohol therapy 100% represents the mean of the pre alcohol (oliguric) values



per volume fluid intake. Following alcohol administration the increase in sodium output in the urine was much less than the increase in the urine volume (Fig 2).

The hyponatremia and the decrease in plasma osmolarity, an expression of hemodilution which occurred during the early postoperative period, was correctable by alcohol administration as depicted by Figure 3. Figure 4 represents an illustrative case demonstrating the described changes.

With osmolarity determinations performed on the urine at frequent intervals, dilution of the urine as evidenced by a decrease in urine osmolarity to as low as 130 mOsm/L occurred during the period of diuresis. By collecting the urine samples at periodic intervals the decrease in urine concentration during diuresis is more readily demonstrated than by studying the 24 hr collection as one sample.

DISCUSSION

A transient disturbance in the metabolism of water and electrolytes is a common phenomenon during the immediate postoperative period. However, in some cardiac patients these changes are exaggerated¹ and present a clinical problem. It is this group of patients that develop the hyponatremic syndrome with oliguria, water retention, and a concentrated urine in the face of hemodilution. To correct this metabolic imbalance the use of alcohol has been effective.

The patients studied developed hyponatremia and hypochloremia between the second and fourth postoperative days. Those patients followed

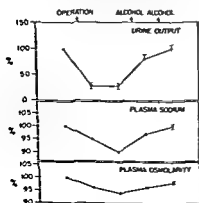


Fig 3 Diagram demonstrating the electrolyte dilution during oliguria and the correction that corresponds to the diuresis. 100% represents preoperative values.

release of antidiuretic hormone from the posterior pituitary^{2 5 10 11} and was reported to produce diuresis in the state of water intoxication⁸ the use of alcohol in the treatment of postcommisurotomy hyponatremia was explored

METHOD

Serum and urine electrolyte measurements and plasma and urine osmolality determinations were followed in all patients scheduled for mitral commissurotomy. Daily fluid intake and output data were obtained. When the hyponatremic syndrome occurred the patient was given 20 to 50 ml of absolute alcohol on the 2nd, 3rd, or 4th postoperative day. The alcohol was given in fruit juice by mouth, or if nausea was present it was given as a 5 per cent intravenous infusion in 5 per cent dextrose in water. The postoperative fluid intake was maintained between 1500 and 2000 ml per day including the alcohol.

Blood samples were analyzed for serum sodium, potassium and chloride concentrations and plasma osmolality. The 24 hr urine collections were measured for specific gravity, osmolality, and content of sodium and potassium. When possible the osmolality of the urine was measured every 2 to 4 hr.

The patients were maintained on the same low salt diet that they received preoperatively. No supplementary salt was given orally or parenterally.

RESULTS

Five patients of the 21 followed developed the postvalvulotomy hyponatremic syndrome. In these 5 patients there was a decrease of 7 to 20 mEq/L in the serum sodium concentration and a decrease of 6 to 20 mOsm/L in the plasma osmolality from their preoperative values. Their urine was concentrated and the volume was less than 500 ml per 24 hr period. Following alcohol administration a diuresis occurred with the 24 hr urine volume being 2 to 6 times the pre alcohol 24 hr volume. The postoperative oliguria and the response to alcohol administration are graphically demonstrated in Figure 1. The *ad libitum* preoperative fluid intake was greater than the fluid intake allowed after surgery. Thus the increase in urine 24 hr volume to 100 per cent of the preoperative value represents a greater urine output

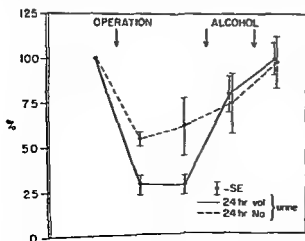


Fig 1 Diagrammatic illustration of the postoperative oliguria and response to alcohol. 100% represents the preoperative value. Each point depicted between operation and alcohol therapy represents the mean value for one half the interval between surgery and therapy (2 to 4 days) to demonstrate the constancy of the oliguric state.

(20 to 50 ml) there was a marked increase in the urinary output with a return of the plasma osmolality, serum sodium and serum chloride to normal. Studies of urinary electrolyte excretion during the phase of increased urinary output suggests a true water diuresis with conservation of sodium. The possible mechanism of this phenomenon is discussed. These studies indicate that alcohol is an effective therapeutic tool in the management of the postcommisurotomy hyponatremic syndrome. The importance of fluid restriction following mitral surgery is stressed.

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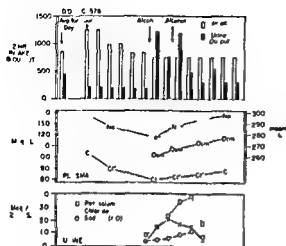


Fig. 1 A case illustrating the occurrence of the hyponatremic syndrome and recovery following alcohol therapy

but not included in the data showed no tendency to develop this syndrome after the fourth postoperative day. In the patients developing the hyponatremic syndrome the administration of 20 to 50 ml absolute alcohol produced a diuresis within 12 hr. The low sodium content of the large urine volume following alcohol administration indicated that the body was retaining sodium and losing water. In these patients the result of the diuresis was a return of serum sodium and chloride and plasma osmolality to normal. The rise in the serum chloride concentration paralleled the change in serum sodium but at a slower rate of recovery. Clinical improvement in these patients was apparent immediately following their diuresis.

It is suggested that this effect of alcohol in inducing a diuresis is due to its inhibition of the release of antidiuretic hormone from the posterior pituitary. It is apparent that the inhibition of the posterior pituitary would have to be of high efficiency since van Dyke¹ has demonstrated that the human posterior pituitary contains about 15,000 m μ of antidiuretic hormone and that the human requires only 0.5 to 2.0 m μ to cause antidiuresis.

Gruer and associates² suggested that the oliguria produced by the positive pressure breathing experiments of Drury *et al*³ and the diuresis occurring during negative pressure breathing could be related to stretch receptors in the auricles and great veins within the thoracic cavity. He further postulated that the antidiuretic hormone could be involved in this phenomenon. Based on these experiments it has been suggested by Sieker⁴ that hyponatremia following mitral valvulotomy may be related to reduction of pressure in the left auricle by the valvulotomy. This may explain why the electrolyte imbalance following mitral surgery is more pronounced than that seen after non-cardiac surgery.

One of the most important features that has become apparent during this study is the necessity of fluid restriction following mitral surgery. Since our first patient was treated with alcohol there has been more rigid control of postoperative fluid intake. As a result there has been an apparent decrease in the frequency and severity of the syndrome.

SUMMARY

Five postvalvulotomy patients had a concentrated urine of low volume, low serum sodium, low serum chloride and electrolyte dilution as evidenced by decreased plasma osmolality. After the administration of absolute alcohol

It has been noted in the literature that a single silk strand strutted across the cavity of the left ventricle soon acquires a smooth endothelial like covering. With this in mind the thought occurred that perhaps nylon would act in the same way and such was the case. The problem of creating a valve like flap was met by fashioning a frame of Ligiloy and covering it with nylon.

Ligiloy* is an alloy developed by the Elgin National Watch Company that has several properties which make it ideal for this purpose. First it is inert in the body second the characteristics of the alloy are such that it will maintain its spring properties for a period far exceeding normal human life expectancy third it can be shaped in a soft pliable state and with simple working and heating is made to acquire spring properties. The frame is made of flat stock which limits lateral motion of the prosthesis even though anchored at a single point. Thus directional motion is obtained with uni-point fixation.

METHOD

In working out a technique for insertion prostheses of various shapes were placed in the mitral area of 65 dogs and the aortic area of 45 dogs.

In the mitral a malleable introducer is passed through the wall of the left

Fig. 21 Four month mitral prosthesis



Fig. 21b 180X Three month aortic prosthesis. The black braid is silk suture the grey braid is nylon.



Material and technical advice through the courtesy of Mr. Thomas H. Green, Elgin National Watch Co. Elgin, Ill.

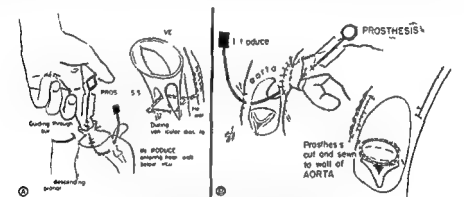
SPRING VALVE PROSTHESIS FOR THE CONTROL OF VALVULAR INSUFFICIENCY*

JAMES H WIBLE, LYLE F JACOBSON PRILSCOTT JORDAN JR.,
AND CHARLES G JOHNSTON

In recent years many approaches to the surgical alleviation of valvular insufficiency have been tried. Some have been used in selected clinical cases, some have never come to clinical trial, none have met general success. The multitude of approaches attest to the lack of a satisfactory method of control.

Despite the type of lesion valvular insufficiency results from the absence of effective coapting valvular tissue. It has been our working premise that except for the occasional circumstance a prosthesis is necessary to cover this deficient area. In order to create a workable device arbitrary criteria were set up which we felt should be met.

A prosthesis should 1) control insufficiency 2) be easily inserted 3) move and continue motion 4) be self repairing i.e. grow living tissue 5) not obstruct the normal directional flow of blood 6) not embolize or form clot that can embolize 7) be tolerated within the circulation i.e. not extruded as a foreign body and 8) not alter blood elements including clotting mechanisms.



Spontaneous mitral insufficiency
with SPRING VALVE

Dog no 171

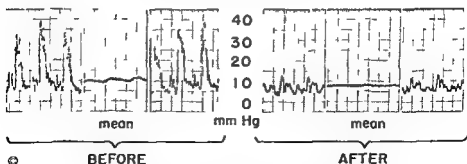


Fig 1a Technique for insertion of mitral prosthesis
Fig 1b Technique for insertion of aortic prosthesis
Fig 1c Left atrial pressures obtained by direct puncture before and after insertion of mitral prosthesis

*From the Department of Surgery, Wayne State University College of Medicine, Detroit Receiving Hospital and the Dearborn Veterans Administration Hospital. Aided by a grant from the Michigan Heart Association, the Research Corporation of the Detroit Receiving Hospital and the Veterans Administration Hospital.

EXPERIMENTAL PROBLEMS IN THE CONSTRUCTION OF SUBCORONARY PROSTHETIC AORTIC VALVES*

W C MAITIE W B SUMMERS AND B EISEMAN

The definitive surgical correction of aortic valvular disease awaits the development of a suitable prosthetic valve that can be inserted beneath the coronary ostia. Valves placed in the aorta distal to the coronary ostia prevent regurgitation of a part of the cardiac output and thereby partially relieve the increased left ventricular strain but only if placed beneath the coronary ostia can they serve the other function of the aortic valve—namely to divert aortic blood into the coronaries during cardiac diastole.

Although a completely successful valve has not yet been achieved a report of experience in the design and insertion of subcoronary prosthetic aortic valves in 53 dogs seems warranted in order to define the technical problem and to describe various procedures that have been employed in its attempted solution.

METHOD

Operative Exposure Surgical exposure of the aortic valve is possible either through the left ventricle or via an incision in the base of the aorta.

In 7 animals various modifications of the left transventricular approach were utilized but exposure of the annulus was inadequate due to limitation of the incision by descending branches of the left coronary artery. This approach was therefore abandoned.

A more direct and satisfactory approach to the aortic valve is via a longitudinal incision in the base of the ascending aorta as has been described by others.^{1, 2, 3} Certain anatomic features of this area are of peculiar importance in the placement of subcoronary prosthetics and bear emphasis. The root of the aorta is a friable easily torn structure that lies buried in the myocardium and is surrounded by vital structures. It is best approached by retracting the right atrium laterally thus exposing the right anterolateral surface of the aorta where an aortotomy incision can be extended to the annulus without involving adjacent structures. Elsewhere about the circumference of the base of the aorta longitudinal incisions are limited inferiorly by myocardium.

By placing tension on 2 traction sutures placed in the annulus good exposure of the entire area is obtained. The tissues immediately above and below the annulus are friable so that sutures must be placed accurately only in the annulus which is firm elastic and holds stitches well. On every side lie vital structures that must not be enclosed with sutures.

Perfusion In 4 animals hypothermia (27°C) with cardiac inflow outflow occlusion has been utilized⁴ but excision and meticulous replacement of the aortic valve with a prosthetic substitute requires a more prolonged period of open cardiotomy than is thus provided. Cross clamping of the ascending aorta and body perfusion with oxygenated blood is therefore required. In 3 cases reservoir oxygenated blood was perfused above the clamped aorta.

From the Department of Surgery, University of Colorado School of Medicine and the Denver Veterans Administration Hospital.

ventricle, through the mitral orifice and out the atrial appendage (Fig 1a). The prosthesis is attached and drawn retrograde into position below the incompetent mitral leaflet. The effect on the jet of insufficiency is readily palpable. A similar technique (Fig 1b) can be used in the aortic area utilizing a side arm sutured to the aorta.

RESULTS

In the process of placing the mitral prosthesis a few animals were found to have spontaneous organic mitral insufficiency. The jet of insufficiency was stopped in these animals as well as in animals with induced insufficiency (Fig 1c). In the aortic area no spontaneous insufficiencies have been found and many methods have been tried to create an aortic insufficiency that is anatomically comparable to that occurring in humans. To date no method has been successful although hemodynamic aortic insufficiency is easy to create by many methods. It can be demonstrated that there is no obstruction to the out flow tract.

The fate of the prosthesis placed in the circulation has been closely followed. A few minutes after insertion the interstices of the nylon become filled with fibrin. In about 18 hr tissue begins to grow onto the shaft of the prosthesis from the endocardium and the frame is completely covered in 12 to 14 days with a smooth glistening endothelial like substance (Fig 2a). Microscopically it is noted that the nylon is invaded by fibrous tissue which is more dense at the surface (Fig 2b). After maturation there appears to be little or no alteration in the tissue with time. The metallic frame can be seen fluoroscopically and motion up to $2\frac{1}{2}$ yr is noted. In the living animals there has been no sign of emboli and in animals sacrificed up to 22 mo there has been no clot on the prosthesis itself or on adjacent endocardium. The reticulocyte counts have not been altered and Lee White clotting times have been normal.

DISCUSSION

The valvulogenic properties of this nylon covered Ligiloy frame are demonstrated. The prosthesis stops insufficiency in the acute preparation in animals. The internal resistance of the spring gives shape to the flap and also prevents the valve from becoming stuck to the wall of the heart or aorta. Unipoint fixation not only makes the insertion easier but minimizes annular eddies which could begot fibrin deposits which could embolize.

The technique of insertion in the mitral area has proven quite simple and safe. The aortic approach is a bit more difficult because of the friability of the aorta and the higher pressure in the aorta.

Studies have been carried out on survivors which indicate that a similar procedure is effective in the area of the aortic valve and gives promise of a method of treatment for aortic stenosis and insufficiency.

SUMMARY

1. Criteria for valvular prosthesis have been formulated.
2. A spring valve is described.
3. Efficiency and valvulogenic properties are discussed.
4. Application to the treatment of aortic stenosis is considered.

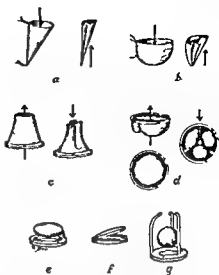


Fig 2 Types of subcoronary aortic valves employed a) Dixie Cup valve b) Rounded Dixie Cup valve in plastic ring seat c) Windsock valve d) Tricuspid valve e) Neumann Bulky Polynog valve f) Flap valve g) Bill valve

more places and the apex fixed to the antero medial aspect of the left ventricle immediately below the first branch of the anterior descending branch of the left coronary artery. In such a location the prosthetic valve does not interfere with the aortic leaf of the mitral valve. During systole the cone collapses allowing unimpeded blood flow into the aorta. During diastole the valve pops open the cone fits snugly into the annulus and regurgitation is prevented.

A total of 21 such valves have been tested—13 with a single layer of nylon and 8 of fused nylon and polythene. The valve was variously fixed to the annulus with a single suture (4 animals) with 2 opposed sutures (3 animals) and with a running suture one half way around the annulus (14 animals).

The longest survival with the use of such conical valves as a replacement after total excision of the aortic leaflets has been 1 hr. These are easily seated, theoretically will be replaced by the host tissue and minimally obstruct ventricular outflow, but there is an almost insuperable degree of fatigue on the plastic cloth and thrombosis occurs in the acute angle of the apex when emptying is not complete.

Type 2—Rounded Dixie Cup Valve (Fig 2b) In order to avoid thrombosis in the apex of the conical valve a cloth valve of similar design but with a rounded bottom has been employed in 2 instances. Such valves shaped much like an aortic leaflet do not thrombose but are more difficult to seat and there have been no long term survivals.

Fused nylon polythene valves have been employed in both instances but this makes the valves more bulky and complicates the problem of stress and tissue fatigue.

Type 3—Wind Sock Valve (Fig 2c) A nylon cloth valve shaped like a wind sock with the base sutured to the annulus and the free end extending into the aorta above the coronary ostia has been employed in one animal. In cardiac diastole the valve collapses and the outflow tract is obliterated. The unsatisfactory features of this valve were its tendency to turn inside out under severe pressure and the partial obliteration of the coronary ostia when the valve collapses during cardiac diastole.

Type 4—Tricuspid Valve (Fig 2d) Insertion of a tricuspid nylon cloth

and in 13 animals chemically induced cardiac arrest using potassium citrate^{6, 7} was employed

In the other 33 animals the ascending aorta was clamped and the remainder of the body perfused with oxygenated blood utilizing the Lillibridge-Wahl technique⁸ and an oxygenator of our own design

In order to provide coronary perfusion while the root of the aorta and the coronary ostia are excluded from the circulation a #18 polythene catheter attached to a side arm of the pump oxygenator has been inserted into the left coronary ostium (Fig 1) A small bulbous tip was fashioned on the end of the catheter to facilitate its insertion into the proximal left coronary artery and to avoid puncturing this fragile vessel The small catheter does not obstruct the surgical field and provides adequate coronary perfusion during the period of open left cardiectomy

In general it would seem that chemically induced cardiac diastole combined with perfusion of the aorta above the occluding clamp is the optimal arrangement If coronary perfusion is indeed necessary⁹ it can be provided by a separate perfusion catheter

Air embolism has not proven to be a serious problem With the coronary ostia exposed through the aortotomy incision there is no evidence of blood flow in the coronary vessels and consequently air is not introduced into the coronary system At the time of aortotomy closure the ventricle is filled by blood from the coronary catheter as it is withdrawn from the ostium and the occasional small air bubbles found in the coronary vessels pass through the coronaries as soon as the infusion pressure of the pump is directed into the coronary vessel after release of the aortic clamp

Valve Design Nylon mesh has been utilized in the construction of the cloth valves in this study In 8 animals a layer of polythene film has been fused to the mesh¹⁰ in order to make it impervious to blood and therefore to make the valve immediately competent All solid valves and valve rings have been made of polyester resin plastic No attempt has been made to develop an ideal plastic for these valves This problem must await their more successful design

Immediately prior to the insertion of the various prosthetic valves the aortic valves were routinely excised with scissors under direct vision

Type 1—Dixie Cup Valve (Fig 2a) This valve is made of nylon cloth cut in the shape of a cone with the open end attached to the annulus in one or

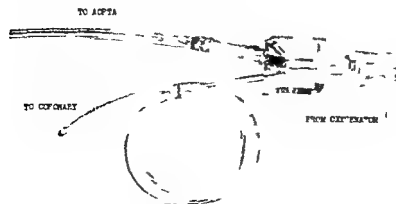


Fig 1 Bulb tip side arm catheter from pump oxygenator used for perfusion of left coronary artery

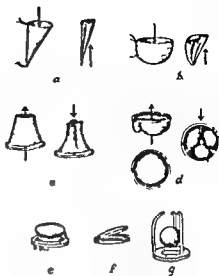


Fig 2 Types of subcoronary aortic valves employed a) Dixie Cup valve b) Rounded Dixie Cup valve in plastic ring seat c) Windsack valve d) Tricuspid valve e) Neumann Bulley Polywog valve f) Flip valve g) Bill valve

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Type 4—Tricuspid Valve (Fig 2d) Insertion of a tricuspid nylon cloth

valve similar in design to the normal aortic valve has been attempted in 14 animals. The 3 round bottom cusps are fused to each other and sutured circumferentially to the annulus. The cusps have been seated in a plastic ring sutured to the annulus in 4 animals. The cusps of these valves must in some way be fixed below the annulus yet impingement on either the aortic leaf of the mitral valve or on the interventricular septum must be avoided. As utilized in this study the cusps materially encroached upon the aortic outflow tract during systole.

Type 5—Neumann Bailey Polywog Valve (Fig 2e) A modification of the Neumann Bailey flap ball valve¹ has been employed in 2 animals with a plastic seating ring sutured to the annulus. These valves produce appreciable aortic stenosis, and their seating on the annulus also interferes with blood flow into the coronary ostia.

Type 6—Flap Valve (Fig 2f) A circular lucite disc of a diameter slightly less than that of the annulus and hinged to a plastic ring seated in the annulus was inserted with but temporary success in 6 animals. This type of valve is easily placed and produces a minimum of aortic stenosis.

Type 7—Ball Valves (Fig 2g) Ball valves of various design have been employed unsuccessfully in 1 case. The success of the Hufnagel ball valve¹² in the descending aorta suggests its use in the subcoronary region, but there are difficulties in such an adaptation, for unless provision is made for enlarging the diameter of the aorta immediately above the annulus where the ball is seated, there will be serious aortic stenosis.

DISCUSSION

A successful subcoronary aortic valve has not as yet been achieved. The surgical approach to the area is feasible. Adequate perfusion both of the myocardium and the remainder of the body can now be attained for the time required for excision of the aortic valve and its prosthetic replacement. The main difficulty therefore lies in the proper design of the valve. If it is to be collapsible it should eventually be replaced by host tissue of an equally flexible nature in order to obviate the ultimate insuperable problem of finding a plastic that will not disintegrate under such conditions. If it is to be a ball valve the cross sectional area of the outflow tract immediately distal to the seated valve must be sufficiently large to avoid stenosis.

Valve placement is most easily accomplished by attaching the valve to a plastic ring which in turn is sutured to the aortic annulus. Since the annulus moves and changes its shape during the cardiac cycle such a plastic ring should also be flexible. An incompletely circular ring best provides for such expansion and contraction.

SUMMARY

1. Although a successful prosthetic subcoronary aortic valve has not yet been achieved, our experience in the design and placement of 7 types of such valves in a series of 53 dogs is reported.

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THE SURGICAL PRODUCTION OF CHRONIC PROGRESSIVE MITRAL INSUFFICIENCY IN DOGS*

SAM J KUYKENDALL, F. HENRY ELLIS, JR., JOHN H. GRINDLAY
AND EARL H. WOOD

A practical method of creating mitral insufficiency in animals with subsequent survival is desirable in order that hemodynamic changes associated with the condition may be studied and various surgical techniques for its correction tested.

Many investigators have produced acute mitral regurgitation experimentally by inserting instruments through the left atrial appendage or the left ventricular wall in order to cut the cusps of the valves sever chordae tendineae or excise portions of the cusps. Usually these procedures produce such extensive valvular damage that the degree of regurgitation is difficult to control and prolonged survival of the animals has been rare.

Haller and Morrow¹ have been able to produce a more controlled degree of mitral insufficiency with prolonged survival in 1 of 15 dogs by selective cutting of the main supporting chordae to the cusps through a closed ventricular approach. Crawshaw, Vetter and Wilson produced regurgitation by suturing the chordae of the posterior cusp back to the ventricular wall and obtained prolonged survival in 6 of 11 dogs. The chordae were selected

*Mayo Clinic and Mayo Foundation, Rochester, Minnesota. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

valve similar in design to the normal aortic valve has been attempted in 11 animals. The 3 round bottom cusps are fused to each other and sutured circumferentially to the annulus. The cusps have been seated in a plastic ring sutured to the annulus in 1 animal. The cusps of these valves must in some way be fixed below the annulus yet impingement on either the aortic leaf of the mitral valve or on the interventricular septum must be avoided. As utilized in this study, the cusps materially encroached upon the aortic outflow tract during systole.

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RESULTS

This report concerns a consecutive series of 29 dogs in which mitral insufficiency was created by this technique. There was one operative death due to cardiac arrest. Six deaths were due to cardiac failure and acute pulmonary edema. These occurred from 7 to 51 days after operation. The over-all prolonged survival rate for this series was approximately 75 per cent. The procedure has been performed subsequently in a number of dogs with an even higher survival rate.

The degree of regurgitation had been graded at the time of creation in the 22 survivors as mild in 1, moderate in 12, and marked in 6. Of the 1 dogs with mild mitral insufficiency, 3 presented murmurs postoperatively, and only 1 of these showed significant dyspnea or gave roentgenologic evidence of cardiac enlargement. At re-exploration 76 to 120 days after creation, the degree of regurgitation was increased in 1, unchanged in 1, and decreased in 2. In none of these animals with mild insufficiency did any dilatation of the mitral ring develop.

In contrast, all of the 12 animals with moderate regurgitation presented systolic murmurs and exertional dyspnea, and two-thirds showed significant limitation of activity or gave roentgenologic evidence of cardiac enlargement.

Furthermore, all of the 6 animals in which regurgitation at the time of its creation was of marked degree presented systolic murmurs immediately postoperatively, and the murmurs persisted. Also, all of these dogs were dyspneic on exertion, were limited in their activity, and gave roentgenologic evidence of cardiac enlargement.

The 18 dogs with moderate or marked regurgitation were explored surgically 7 to 27 weeks after creation of the mitral insufficiency. The amount of regurgitation as determined by palpation was unchanged in 3 of the dogs and was increased in intensity in 15 (83 per cent) of the 18. Intratrial palpation gave the gross impression of definite secondary dilatation of the mitral ring in 17 (94 per cent) of the 18 dogs. In all 18 dogs the left atrium and left ventricle were enlarged, and in those dogs in which regurgitation had been greatest the right ventricle was enlarged as well.

In 11 dogs the intensity of the systolic murmur had increased before exploration. In all of these 11 the mitral rings were found to be dilated and the degree of insufficiency was found to be greater than had been created originally. The observation suggests that in this series of dogs the increase in the intensity of the murmur was indicative of the development of secondary dilatation of the annulus. The impression obtained by palpation that such dilatation occurred in 17 of the 18 dogs demonstrates the progressive pathologic and physiologic changes that develop in dogs with prolonged mitral insufficiency.

Simultaneous left atrial and left ventricular pressures were recorded for some of the dogs. The changes in contour and pressure closely paralleled those seen in human beings with mitral insufficiency. The left atrial mean pressure consistently was increased, the average being 11.3 mm. Hg as compared with the preregurgitation average of 3.0 mm. There was prominent elevation of the V wave.

Indicator-dilution studies also were performed in some instances. The dye

and the suture carrier guided by a finger inserted through the left atrial appendage

METHOD

Anatomic study of the region of the mitral valve in fresh specimens obtained at necropsy suggested the possibility of creating mitral regurgitation by hooking the chordae of the mural (posterolateral) cusp and tacking them to the left ventricular wall. A direct left ventricular approach was used leaving the atrial appendage intact for later use in gaining access to the valve region for applying corrective procedures. A small blunt nerve hook was inserted through a small epicardial incision at a point 1.5 cm anterior to the left marginal coronary vessels and 1 cm below the circumflex coronary vessels (Fig 1). Use of this site avoided injury to the larger coronary vessels and damage to the papillary muscles and the leaflets. Chordae which supported the lateral one third to one half of the mural (posterolateral) cusp were engaged with the hook. Then while steady traction on the instrument held the tendons against the endocardium a heavy cotton suture was passed through the ventricular wall and back encircling the ensnared chordae. A modified aneurysm needle was used to pass the suture. After careful withdrawal of the hook disengaging the chordae without severing them the ends of the suture were tied over a small square of compressed polyvinyl (Ivilon) sponge placed on the epicardium. The extent of immediate ballooning of the atrial appendage and the intensity of the palpable thrill over the left atrium indicated the degree of regurgitation produced.

This method has the advantage of producing shortening of the chordae and retraction of the mural cusp simulating the condition usually seen in human beings with rheumatic mitral insufficiency except that the resulting defect is in the region of the anterolateral commissure rather than that of the posteromedial commissure. In the dogs that have been killed subsequently the ensnared chordae and a small portion of the retracted cusp have been found to be firmly adherent to the endocardial surface of the left ventricle.

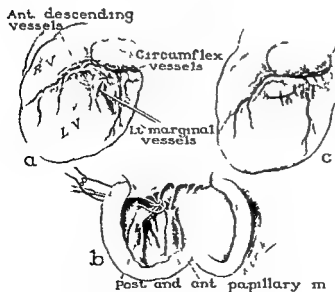


Fig 1 a The site of the incision the hook engaging chordae is within the left ventricle b The hook holding chordae of the posterolateral cusp against the endocardium during placement of the encircling suture c The position of the finished suture The polyvinyl sponge over which the suture is tied is not shown in the drawing

in order to study changes in the pulmonary artery. This paper will present this study.

METHOD

Mitral stenosis was produced in 8 dogs by the previously described method.¹ The dogs were anesthetized with intravenous pentobarbital sodium 2 wks before and from 2 wks to 13 mo after production of experimental mitral stenosis. With the animal supine, cardiac catheters were introduced into the pulmonary artery and vein. Pulmonary artery, pulmonary vein, and intrathoracic pressures were recorded with standard resistance wire pressure transducers (Statham Strain Gauges). Zeros were arrived at by visualizing the tips of the catheters fluoroscopically and exposing the strain gauges to atmospheric pressure at that level. Pressures were obtained in all 8 dogs just before sacrifice. The circumference and the diameter of the mitral valve were measured in the dogs after sacrifice. Since the orifices were circular, the area was calculated from the circumference. Using Rubner's constant in Meeb's Formula 11.2 (wt in gm) to calculate surface area, the valve area was then expressed per square meter of body surface area. The lungs were sectioned and prepared for microscopic examination.

RESULTS

Four of the dogs sacrificed showed no appreciable narrowing of the mitral valve. The remaining 4 dogs had a moderate to a marked degree of experimental mitral stenosis.

The dogs with no mitral narrowing had an average pulmonary vein pressure of 7.5 mm Hg and a pulmonary artery pressure of 16.5 mm Hg. In the 4 animals with marked narrowing of the mitral opening, the pulmonary vein pressures averaged 16.5 mm Hg and the pulmonary artery pressure averaged 25 mm Hg (See Table 1). The lung sections were read by Dr. John F. Noble, Pathologist at Ancker Hospital. They were also seen by Dr. Jesse Edwards of the Mayo Clinic. Both of these men were shown the slides without any knowledge of the dog from which they came. Each was able to distinguish changes in the small pulmonary arteries of the 4 dogs with the most mitral narrowing (See Table 2).

These changes consisted for the most part of medial thickening but with occasional vessels showing some intimal thickening.

Table 1 Average Pressures in Pulmonary Artery and Pulmonary Vein

DOG NUMBER	WEIGHT	VALVE SIZE cm ² /m ²	PULMONARY VEIN	PULMONARY ARTERY
1	43#	5.8	8	12
2	45#	1.3	8	13
3	44#	4.6	10	16
4	43#	2.5	5	19
5	46#	9	20	24
6	40#	.8	15	21
7	43#	4	23	32
8	39#	.5	18	25

(Evans blue) was injected into the left ventricle and simultaneously recorded at the left atrium and the femoral artery. The dye constantly appeared in large quantities in the left atrium before it appeared in the femoral artery.

SUMMARY AND COMMENT

A method is presented of producing mitral insufficiency in dogs which is fairly well controlled in degree and also compatible with prolonged survival. The anatomic defects produced—shortening of chordae tendineae, retraction of the cusp, and secondary dilatation of the mitral ring—are similar to the pathologic findings in human beings with mitral insufficiency of rheumatic origin. The hemodynamic changes and the clinical manifestations of murmurs, dyspnea, and cardiac enlargement are comparable to those of patients who are suffering from this disease. This experimental preparation may be of value in the further investigation of hemodynamic changes and of methods of correcting mitral insufficiency.

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PULMONARY ARTERY CHANGES IN MITRAL STENOSIS*

An Experimental Study

DONALD W. HANNON, ALLAN FERRIN, FRANCIS HADDY,
JOSEPH L. SPRAFKA AND IVAN D. BARONOVSKY

As more and more of the long term results of mitral commissurotomy are received, it is obvious that the operation is of marked benefit in about 70 per cent of patients operated upon. One of the obstacles to a successful result of this operation has been fixed organic changes that have occurred in the pulmonary artery as a result of prolonged pulmonary venous hypertension. Adequate experimental studies of pulmonary artery changes have been hampered in the past by the lack of a good method for producing experimental mitral stenosis. At the 1951 Forum meeting two of us¹ studied the problem extensively and reported an experimental method of producing mitral stenosis. The method consisted of inversion of the left auricular appendage across the mitral ring above the valve. Very marked narrowing of the mitral orifice was obtained. Using this method the hemodynamics of mitral stenosis were studied.^{2,4,5} In addition a group of 8 dogs was allowed to live for 3 to 4 yr.

*From the Department of Surgery, University of Minnesota and the Surgical Research Laboratories of Ancker Hospital, St. Paul, Minn.

THE HEART

in order to study changes in the pulmonary artery This paper will present this study

METHOD

Mitral stenosis was produced in 8 dogs by the previously described method.¹ The dogs were anesthetized with intravenous pentobarbital sodium 2 wks before and from 3 wks to 43 mo after production of experimental mitral stenosis. With the animal supine cardiac catheters were introduced into the pulmonary artery and vein. Pulmonary artery pulmonary vein and intrathoracic pressures were recorded with standard resistance wire pressure transducers (Statham Strain Gauges) Zeros were arrived at by visualizing the tips of the catheters fluoroscopically and exposing the strain gauges to atmospheric pressure at that level. Pressures were obtained in all 8 dogs just before sacrifice. The circumference and the diameter of the mitral valve were measured in the dogs after sacrifice. Since the orifices were circular the area was calculated from the circumference. Using Raubner's constant in Meek's Formula $11.2 (\text{wt in gm})$ to calculate surface area the valve area was then expressed per square meter of body surface area. The lungs were sectioned and prepared for microscopic examination.

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Table 1 Average Pressures in Pulmonary Artery and Pulmonary Vein

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2	45#	1.3	8	18
3	44#	4.6	10	16
4	43#	2.5	5	19
5	46#	9	20	24
6	40#	8	15	21
7	43#	4	23	32
8	39#	5	18	25

Table 2

DOG NUMBER	VALVE SIZE cm ² /m ²	TIME STENOSIS PRESENT	THICKENING OF SMALL ARTERIES
1	5.8	3 yrs 2 wks	0
2	1.3	3 yrs 2 wks	0
3	1.6	3 yrs 3 mo	0
4	2.1	3 yrs 1 mo	0
5	.9	3 yrs 1 mo	1 resent
6	.8	3 yrs 2 wks	1 resent
7	.1	3 yrs 7 mo	1 resent
8	.5	3 yrs 8 mo	Present

We are deeply indebted to Dr. John F. Noble of Ancker Hospital and Dr. Jesse Edwards of the Mayo Clinic for their help in examining these lung sections.

There seemed to be a good correlation between mitral orifice size and the small artery changes. No changes were noted where the valve size was over 1 cm. in diameter.

DISCUSSION

The changes noted in the small pulmonary arteries of the dogs with experimental mitral stenosis are moderate in amount but the fact that they occur is most important. It would have been interesting to see how soon these changes would regress with reopening of the narrowed mitral area.

It may be surprising to find that only moderate increase in pulmonary artery pressures was observed in dogs with arterial changes. In a previous publication we have shown that pulmonary resistance is inversely related to the pulmonary vein pressure. In all probability pressures in the pulmonary artery will rise to high levels not as a result of high pulmonary vein pressures but as a result of very advanced organic changes in the pulmonary artery. In these animals the changes are present but haven't advanced to that stage. Therefore little emphasis can be placed on pulmonary artery pressures as an indication of the presence or absence of small artery thickening. The early development of these changes probably represents a very strong argument in favor of the early operation of mitral stenosis. Pulmonary hypertension once it is fully developed may represent progression of the arterial changes to an irreversible state. Certainly the changes which are noted in our experimental group could possibly be expected to revert as opposed to the changes seen with well established pulmonary hypertension. Even if they remained as is it can be stated that the work load of the right ventricle would not be increased any further if operation were successfully accomplished. A word might be interjected at this time regarding the procedure of producing the stenosis. The ease with which narrowing of the mitral orifice can be accomplished by this method suggests the possibility of using a variation of the technique in the treatment of mitral insufficiency.

CONCLUSIONS

1. Effective chronic narrowing of the mitral ring can be accomplished easily by the method previously described.

2 Dogs with moderate to marked experimental narrowing of the mitral area developed small artery changes consisting of medio thickening and occasional intimal thickening after 37 to 14 months

3 The small artery changes developed in the absence of any marked elevation of pulmonary artery pressures

4 It would not seem advisable to wait for elevation of pulmonary artery pressures as an indication for surgery in mitral stenosis

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MEASUREMENT OF THE ELECTRICAL IMPEDANCE OF THE HUMAN HEART DURING DEFIBRILLATION*

CHARLES K. KIRBY JOSEPH ENGBERG AND JULIAN JOHNSON

Several years ago we observed that much stronger shocks were apparently required for successful defibrillation of the ventricles of patients with large hearts than of those with hearts of approximately normal size. We then studied the problem of optimum voltage in dogs and found that single shocks of 270 volts were considerably more effective than single shocks of 110 volts in abolishing ventricular fibrillation of 4 min duration.¹ Shocks up to 270 volts caused no grossly visible burns or other ill effects when the duration did not exceed 0.10 sec. On the basis of this evidence a timing device and high voltage transformer were incorporated in our operating room defibrillator. We adopted the policy of administering a high voltage shock of short duration in patients with large hearts rather than beginning with low voltage shocks and working upward as had formerly been our practice. The single high voltage shocks were usually successful.

The apparent need for stronger shocks for larger hearts raised the question as to whether large hearts had greater electrical impedance or whether other factors associated with cardiac enlargement made defibrillation more

*From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia. Supported by a grant from the York County (Pennsylvania) Heart Association.

difficult. No information concerning the impedance of the heart in living humans could be found in the literature.

We therefore designed a defibrillator to measure the cardiac impedance during the application of shocks in patients with ventricular fibrillation. This defibrillator has been used in the operating room for nearly two years.

METHOD

The circuit diagram of the defibrillator used in these studies is shown in Figure 1. The electrodes were made of stainless steel and were 6.4 cm in diameter. They were applied directly to opposite sides of the heart. The handle of each electrode was insulated but the back was not. Moderate firm pressure was used to reduce the distance between the electrodes. An attempt was made to keep the amount of electrode compression relatively uniform and we believe that this was possible since the electrodes were handled by the same operators in nearly every patient. Single 0.10 sec. shocks of from 120 to 230 volts (60 cycle per sec.) were used in most instances and each single shock usually stopped fibrillation. Repetition of the shock was often necessary because fibrillation recurred. Serial shocks were sometimes needed especially after single shocks had been used repeatedly for 30 min. or more.

The defibrillation current was recorded on a Sanborn Model 127 Recorder. The apparatus was calibrated with known impedances and currents. The current passing through the heart and the heart impedance, as reflected in the height of the deflection in millimeters, was determined from a calibration chart.

The recordings made on 28 patients were available for analysis. In 22 of the patients the operative procedure involved the heart or great vessels.

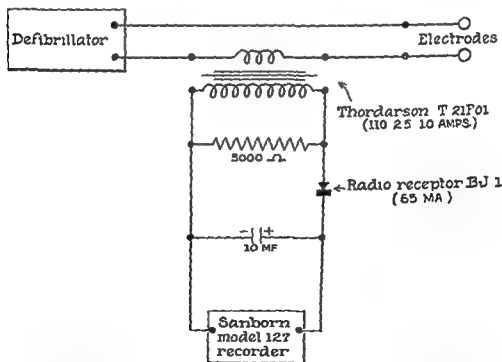


Fig. 1. Circuit used with the defibrillator to measure and record the current passing through the heart. Constructed for us by American Electronics Laboratories, Inc., 641 Arch Street, Philadelphia, Penna.

Four patients had a pulmonary resection 1 a cholecystectomy, and 1 a craniotomy for brain tumor

RESULTS

Of the 28 patients 1 recovered completely and were discharged from the hospital Seven lived from 1 to 36 hr postoperatively and the remaining 17 did not survive the operative procedure Ventricular fibrillation was abolished in every patient In many patients it recurred often repeatedly, but death was due to inability to establish an effective heart beat rather than to intractable fibrillation

The electrical impedance of the hearts of the 28 patients ranged from 20.5 to 49.5 ohms with a median of 35.7 ohms * The current ranged from 2.7 to 8.8 amperes with a median of 5.7 amperes The accuracy of measurement of heart impedance was estimated to be plus or minus 10 per cent and of current plus or minus 5 per cent The heart weight was determined at autopsy in 17 patients The weight varied from 310 to 900 gm with a median of 450 gm There appeared to be little evidence of correlation between heart weight and impedance the correlation coefficient being plus 0.1

DISCUSSION

Mackay, Mooshin and Leeds² found that the average resistance of the living dog heart was about 50 ohms Bertie, Keshishian, Ames and Blades³ confirmed this observation and also studied dead dog hearts which had an average resistance of 97 ohms and dead human hearts which had an average resistance of 93 ohms

In this study we have found that the impedance of large human hearts was not greater and actually appeared to be less than that of live dog hearts which are considerably smaller There appears to be little if any correlation between heart size and impedance This is strikingly illustrated by the fact that the impedance of the largest heart in this series (900 gm aortic stenosis) was only 26.2 ohms which was well below the average for the entire series of 35.7 ohms

Our previous suggestion¹ that a transformer be used to produce voltages higher than the usual house current (110-115 volts) for defibrillation of large hearts received support in this study In many instances shocks of 230 volts were successful after shocks of lower voltage had failed

Since there appears to be no significant correlation between electrical impedance and heart size the need for higher voltages for larger hearts is apparently due to factors other than increased resistance It seems likely that such factors include the disease process itself as well as hemodynamic and metabolic abnormalities which are the cause or the result of cardiac enlargement

CONCLUSIONS

1 The electrical impedance of and the current passing through the human heart was measured during the application of electric shocks of 120 to 230 volts in 28 patients in ventricular fibrillation

In most patients receiving multiple shocks the variation of the heart impedance about the patient's mean impedance was ± 15 per cent (occasionally as high as ± 30 per cent) This was probably due to variations in electrode compression

2 The impedance ranged from 20.5 to 19.5 ohms with a median of 35.7 ohms. It appeared to be lower than that of the living dog heart.

3 The need for high voltage shocks in defibrillating large human hearts appears to be due to factors other than an increase in electrical impedance.

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THE EXPERIMENTAL PRODUCTION OF ARRHYTHMIAS BY SELECTIVE DESTRUCTION OF THE CARDIAC CONDUCTION SYSTEM*

ROBERT W HARRISON, PETER V MOULDER JR, PAUL V HARPER
AND WILLIAM E ADAMS

Present day cardiac surgery has given little attention to the feasibility of correction of rhythm disturbances and conduction system defects. Experimental subjects for the evaluation of medical and surgical therapy of arrhythmias were sought in these experiments by the selective destruction of segments of the conduction system and observation of the rhythm produced.

Physiologic studies of the function of the conduction system by early investigators were frequently performed on perfused heart preparations or as acute experiments on the heart *in situ*. In the following experiments well defined conduction system defects were produced by radiation from beta ray point sources. In contrast to the earlier studies these were gradual in production and chronic in nature, the animals being observed during the period of development of the lesion and for long periods of time thereafter. A histochemical technique was utilized for accurate demonstration and microscopic delineation of the limits of the conduction system and for evaluation of the extent of the lesions produced.

METHOD

Operations were performed on 18 mongrel dogs. The exact details of the operative technique vary depending upon the portion of the conduction system attacked.

*From the Department of Surgery and the Abgonne Cancer Research Hospital, University of Chicago Clinics, Chicago, Illinois. This study aided by grants from the Oscar Mayer Foundation and the Douglas Smith Foundation for Medical Research of the University of Chicago.

The point source radiation was provided by yttrium oxide pellets containing 0.5 to 1.5 mc of ^{90}Y activity. The cylindrical pellets were 0.5 mm in diameter and 1 to 1.5 mm in length. Details for the preparation of yttrium oxide pellets have been described.¹ The radioactive pellets were handled by insertion in lead shielded 17 gauge spinal needles. The tip of the needle was inserted into the tissue to the desired location and the pellet then ejected by means of the obturator of the needle. The radioactive yttrium pellets emit beta radiation which has a half life of 62 hr. and a maximum range in tissue of approximately 1.0 cm. The areas of necrosis produced by these beta ray point sources are sharply demarcated approximately 8 mm in diameter.

Pockets of various material (free pericardial graft free aortic wall homograft and thin polyethylene sheeting) were fashioned to cover generously the area of the right atrium which contained the sino atrial node. The pockets were sutured to the surface of the atrium on the posterior wall at the cavo atrial junction and along the sulcus terminalis with #5/8 silk. Radioactive pellets were inserted in the pockets in contact with the atrial wall spaced at 5 to 6 mm intervals. Five to 8 pellets were used depending upon the size of the animal and the activity of the pellets.

Hypothermia (24 to 26°C) and complete interruption of the circulation with the employment of acetylcholine to render the heart essentially asystolic were utilized to permit performance of a right atriotomy and visualization of the area of the interatrial septum containing the atrio ventricular node.² In the area between the ostium of the coronary sinus and the septum membranaceum 4 to 5 pellets were implanted subendocardially spaced approximately 5 mm apart.

Preoperative and serial postoperative standard and augmented unipolar limb lead electrocardiograms were obtained on all animals.

The histochemical technique utilized to demonstrate and study the location of the specialized muscle tissues that make up the cardiac conduction system depend upon the demonstration of cholinesterase activity.³ Routine histologic studies were also performed. Histochemical studies were performed on a series of normal hearts as well as those hearts in which conduction system lesions had been produced to determine the location and extent of the lesions.

RESULTS

The normal conduction system in the dog's heart can be demonstrated enzymatically. The enzyme is localized in the myofibrils of the specialized cardiac muscle fibers. The boundaries of the conduction system have a sharp outline and no transition is seen between the specialized muscle fibers and the cardiac muscle cells.

The site of the enzymatic reaction representing the sino atrial node was found to be in the crista terminalis. The limits of the sino atrial node were sharply demonstrated by a positive zone which occupied a portion of the posterior wall of the right atrium and extended inferiorly along the sulcus terminalis to a point near the junction of the inferior vena cava with the right atrium.

The atrio ventricular node occupies a position in the lower portion of the interatrial septum between the ostium of the coronary sinus and the septum membranaceum.

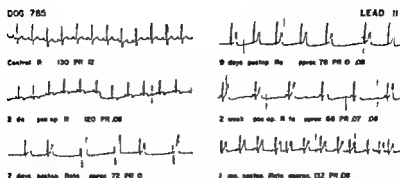


Fig 1 Electrocardiographic tracings following the implantation of five 0.5 mc Y^{90} pellets in the region of the sino atrial node

2 days postop—Essentially normal EKG except for reduction of PR interval from 12 to 08 sec.

7 days postop—Marked arrhythmia P waves absent Intermittent escape beats

9 days postop—Marked arrhythmia P waves seen only occasionally QRS complex of normal configuration

2 weeks postop—P wave constantly present with P R interval reduced to 07 to 08 sec.

1 mo postop—Permanent rhythm established Note decreased amplitude of P wave and decreased P R interval

Sino atrial node Total ablation of the sino atrial node as evidenced by the absence of enzymatic activity histochemically results in only minimal permanent rhythm disturbances and electrocardiographic alterations. In the early postoperative period, rhythm disturbances are quite variable and unpredictable. A nearly constant finding is the diminution in amplitude or disappearance of the P wave which has been observed to be markedly reduced in potential within 24 hr after the implantation of the pellets and has been noted to be absent as early as 48 hr postoperatively. Not uncommonly there is a marked reduction in cardiac rate during this early period. Frequently in the early period there is an absence of the P wave which is associated with a normal appearing QRS complex and a nonphasic arrhythmia in other words a rhythm more sinus than nodal. In those cases where the P wave remains visible the P R intervals show a definite shortening from a control value of 09 to 12 sec to 06 to 08 sec. During the first 2 to 3 wks following the implantation of the radioactive material there may be observed frequently intermittent periods of sino auricular block or auricular standstill with associated beats of nodal origin or so called escape beats. In 2 to 3 wks the permanent pattern is established with a rate approximating that of the control but with decreased amplitude of the P wave and a shortened P R interval. Figure 1 illustrates these electrocardiographic changes.

Atrio ventricular node Radioactive Y^{90} pellets implanted in the region of the atrio ventricular node do not constantly produce an atrio-ventricular heart block. In the animals in which heart block developed the rhythm disturbance was first manifested by a prolongation of the atrio ventricular conduction time represented by a prolonged P R interval. Often this P R interval would be variable in duration before becoming constantly prolonged and later (1 wk) complete heart block would develop with its

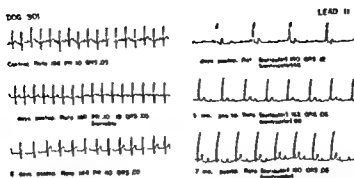


Fig. 2 Electrocardiographic tracings following the implantation of four 1 mc Y^{90} pellets in the region of the atrioventricular node

4 days postop The I R interval is variable ranging from 10 to 19 sec Signifying a fluctuating degree of heartblock

6 days postop A constant first degree heart block with a I R interval of 40 sec Due to the prolonged I R interval the I wave is frequently located within the T wave of the preceding beat

7 days postop Complete heart block

1 mo postop Complete heart block

7 mo postop Complete heart block

typical atrioventricular dissociation Figure 2 shows excerpts of serial electrocardiographic tracings of one animal in which a permanent heart block was produced Complete heart block has been observed to be permanent in those cases in which it developed

DISCUSSION

Rhythm disturbances observed in the early period during progressive destruction of the sinoatrial node i.e. sinoatrial block auricular standstill and frequent escape beats are similar to the changes one would expect with removal of the pacemaker of the heart Other workers basing their conclusions on experiments in which the destruction or functional depression of the sinoatrial node has been relatively sudden felt that the resulting rhythm arose from the coronary sinus or ventricular portion of the atrioventricular node

However the true site of impulse formation in these hearts is not readily apparent Due to the positive character of the P wave it must be concluded that the site of impulse formation in these hearts is located above the coronary sinus and the direction of spread of the impulse over the right atrium is normal Nevertheless the constant decrease in P R interval suggests the location of the site of origin close to the atrioventricular node

It is felt that the slow destructive process used in these experiments simulates changes occurring clinically in most disease processes affecting the sinoatrial node other than embolism or sudden thrombosis of the nutrient vessels The difficulty with which sinoatrial block and auricular standstill are produced by gradual ablation of the sinoatrial node corresponds with the rare clinical observation of such arrhythmias in conditions other than those associated with acute drug toxicity

The inconstancy in our ability to produce heart block by implantation of

the pellets in the region of the atrioventricular node is more than likely dependent upon at least 2 factors. First, as a result of autonomic phenomena it is necessary to affect every fiber of the atrioventricular system to produce a conduction disturbance. Secondly although it is possible to place the pellets quite accurately, slight variations in the location of the atrioventricular node would result in incomplete destruction and consequently no impairment of conduction.

CONCLUSIONS

1. Local beta radiation as produced by small radioactive yttrium⁹⁰ oxide pellets provides a means of gradually creating well localized defects in the conduction system of the heart.

2. Gradual and complete ablation of the sinoatrial node results in transient rhythm disturbances, i.e. auricular standstill, sinoatrial block, and nodal rhythms but almost invariably the permanent rhythm is characterized by an upright P wave of decreased potential and a shortened P-R interval.

The site or origin of the cardiac impulse following ablation of the sinoatrial node is not known.

3. Permanent complete atrioventricular heart block may be produced by application of local beta radiation in the region of the atrioventricular node.

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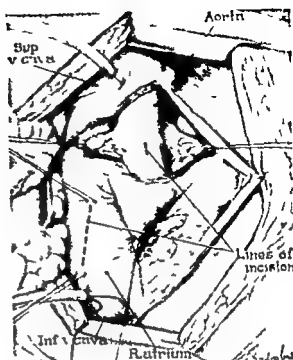
EXPERIMENTAL CLOSURE OF RUPTURED SINUS OF VALSALVA ANEURYSMS*

R. ROBINSON BAKER, HANS ERIK HANSON AND ANDREW G. MORROW

Rupture of a congenital aneurysm of the aortic sinus of Valsalva results in a fistulous communication between the base of the aorta and the right heart. The usual sequelae are progressive cardiac failure and death. A method for the closure of such fistulae was suggested during aortography in a patient with this lesion. A catheter was easily passed from the aorta through the fistula and into the right atrium. The course of the catheter suggested the experimental method described.

*From the Clinic of Surgery, National Heart Institute, Bethesda 14, Maryland.

Fig 1 Operative exposure prior to inflow occlusion. The sites of aortic and atrial incisions are indicated.



METHOD

The prostheses shaped like golf tees were made from compressed polyvinyl (Ivalon) sponge. The diameter of the flattened head was 4 to 5 mm and that of the shaft 2 to 3 mm. They were sterilized by boiling.

Dogs weighing 15 to 20 kg were anesthetized with intravenous pentothal and cooled by immersion to a final rectal temperature of 33 C. The right chest was entered through the fourth intercostal space and traction ligatures placed about the superior and inferior venae cavae. The azygos vein was ligated and the pericardium opened anterior to the phrenic nerve. The right atrial appendage was retracted inferiorly, exposing the root of the aorta. The adventitia of the aorta was excised and retracted to each side by means of silk sutures. Stay sutures were then placed in the body of the right atrium.

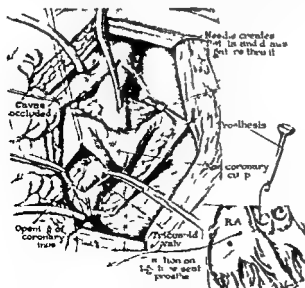


Fig 2 The fistula is created between the aorta and right atrium with a modified Reverdin needle. A heavy ligature is drawn through it and the prosthesis introduced through the aortic incision.

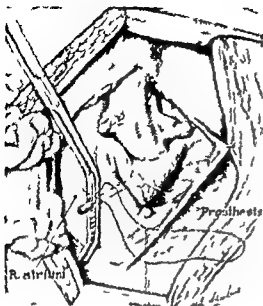


Fig 3 The aortic and atrial incisions are closed the tail of the prosthesis is incorporated into the atrial suture line

Incisions were made in the aorta and right atrium following the application of partially occluding clamps (Fig 1)

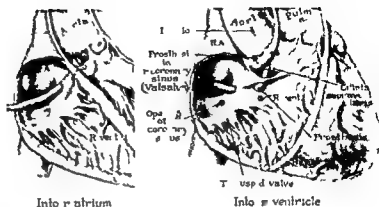
In 10 animals a fistula was created between the non coronary aortic sinus and right atrium Following inflow occlusion the right atrial clamp was removed and the right index finger placed just superior and medial to the opening of the coronary sinus A modified Reverdin needle was then introduced through the aortic incision and advanced into the non coronary sinus until the tip was palpated by the finger within the right atrium A tract was created by advancing the needle through the aortic wall and out into the atrium (Fig 2) A heavy ligature was threaded into the eye of the needle and the needle withdrawn from the aorta The ligature then passed from the aorta through the newly created tract and into the atrium The shaft of the prosthesis was tied to this ligature and the prosthesis pulled into the aorta so that its proximal flattened end lay behind the aortic leaflet The shaft occluded the tract and was brought out through the atrial incision Partially occluding clamps were then reapplied to the aortic and atrial incisions and circulation reestablished These incisions were closed with continuous over and over sutures the tail of the prosthesis being incorporated into the atrial suture line

In 11 dogs, a tract was created between the right coronary sinus and right ventricle In these dogs the index finger was placed under the septal leaflet of the tricuspid valve The Reverdin needle was introduced into the right coronary sinus and advanced into the right ventricle and then into the right atrium at a point just medial to the septal leaflet of the tricuspid valve The prosthesis was introduced so that its head lay behind the right coronary leaflet and its shaft passed from the ventricle across the septal leaflet of the tricuspid valve The tail of the prosthesis was again incorporated into the atrial suture line (Fig 4)

There were three operative deaths in the 21 animals 1 each from anesthetic overdosage distemper and a technical error Aortic and left ventricular catheterizations were performed 1 wk postoperatively The animals were sacrificed at 1 to 4 wk intervals following the operation

Final Position of Prostheses

Fig 4 Final positions of the prosthesis in fistulae created between the non coronary sinus and right atrium and right coronary sinus and right ventricle



RESULTS AND DISCUSSION

There were no manifestations of aortic stenosis or insufficiency in any animals. The aortic and left ventricular catheterizations showed no systolic gradients across the aortic valve and the arterial pressure pulses were normal. At autopsy the aortic valve leaflets were undamaged. The aortic and atrial portions of the prostheses were covered with smooth endothelium.

Lillehei¹ and Bahnson² have recently closed cardio aortic fistulae by suture from the right side during total cardiac bypass. Edwards³ has pointed out that the essential pathologic feature of a congenital aneurysm of the sinus of Valsalva is a lack of continuity between the aortic media and the annulus of the aortic valve. In the method described this continuity is restored by bridging the defect with a prosthesis introduced from the aortic side.

The operation has recently been successfully performed in a 28 year old man in whom an aneurysm of the right coronary sinus had ruptured into the right atrium. This case will be subsequently reported in detail.

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FURTHER STUDIES IN INDUCED CARDIAC ARREST USING THE AGENT ACETYLCHOLINE*

CHARLES K. SHERMAN, THOMAS GEOHEGAN AND CONRAD R. LAM

The advantages of carrying out open heart operations on a motionless heart are obvious. In a completely bloodless and quiet field these procedures are greatly facilitated. The relatively successful use of potassium chloride as a cardioplegic agent was reported by us in 1955.¹ However, resuscitation was not invariably successful and was usually complicated by ventricular fibrillation. Therefore in November 1955 we decided to try the drug acetylcholine although we were aware that Bjork² had not been successful with it in at least one experiment.

The ability of acetylcholine to stop the heart was easily demonstrated in a series of acute experiments. Under endotracheal anesthesia with ether the thoracotomy was carried out on 20 dogs. The pericardium was opened, the ventricle occluded by snares, the ascending aorta occluded and 10 mg/kg of acetylcholine (Acccholine Anglo French Labs) was injected into the proximal aorta where presumably most of it entered the coronary arteries. The commercial preparation was diluted 1:10 with saline solution. Prompt and complete cardiac arrest was produced (Fig. 1). Resuscitative measures were begun after 5 to 10 min. of arrest, the essential step being the perfusion of the coronary arteries with blood to wash out the cardioplegic drug. In 8 animals the perfusion was accomplished by forcing blood from a reservoir through a tube inserted through a subclavian artery, and in 12 animals ordinary cardiac massage was used. Following a total of 31 arrests, resuscitation was attained in all instances.

We then proceeded to use this method of elective cardiac arrest on animals in which the brain was protected by general hypothermia. Two types of intracardiac operations were carried out. In 20 animals partial aortic stenosis was produced by suturing 1 or 2 commissures and in 4 animals ventricular septal defects were made and repaired. Cardiac rhythm was restored in each instance by massage. Twenty-two of these animals survived. 2 died on the third postoperative day of hemorrhage from the aortic incisions. Cir-

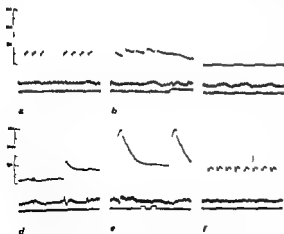


Fig. 1 Simultaneous tracings of the aortic pressure and lead II of the electrocardiogram in a normothermic dog. a normal b effect of caval occlusion and c the signal the injection of acetylcholine into the coronary arteries. d record during complete arrest. The undulations of the electrocardiogram are due to the mechanical respirator. e shows the first ventricular contraction 10 sec after the beginning of the coronary perfusion with oxygenated blood. f improved contractions 4 min later. g normal pressure and electrocardiogram 10 min later.

*From the Division of General Surgery, Henry Ford Hospital, Detroit, Mich. Supported by a grant from the Michigan Heart Association.

dic resuscitation in 5 animals was complicated by ventricular fibrillation which was corrected by 1 or 2 countershocks

During the period of massage the heart passed through a phase of cyanosis and right sided dilatation. As massage was continued the cyanosis gave way to a rapid and dramatic spread of normal color over the heart, spontaneous contractions began and there was immediate emptying of the right side. At this time the electrocardiogram showed the onset of a sinus rhythm. We have since found that this period of myocardial cyanosis and lack of sinus rhythm can be reduced materially by intracoronary injection of small amounts of atropine.

In March 1956 a pump-oxygenator of the type used by Lillehei, De Wall and their co workers became available to us.³ Since that time this method of protecting the brain has been used instead of hypothermia. Following the tightening of the snares around the 2 vena caval cannulas the aorta was clamped between the inflow cannula (through a subclavian artery) and the coronary artery orifices. A solution of acetylcholine (10 mg/kg of body weight) was injected into the proximal aorta and arrest of the heart was immediately produced. The right ventricle was then opened and an interventricular septal defect created and repaired. As the ventricular wall was being sutured the clamp was removed from the aorta, permitting the perfusion of oxygenated blood through the coronary arteries. The first blood from the coronary sinus (containing acetylcholine) was removed from the right ventricle by aspiration. Ventricular contractions frequently appeared within 1 min. of the removal of the aortic clamp. At first the contractions were coordinated but weak and infrequent. As the perfusion was continued, there was increase in the strength and frequency of the contractions and 4 or 5 min. later the aortic pressure was sufficiently well maintained by the heart itself so that the pump oxygenator could be discontinued.

Twenty five dogs have been operated on by this technique. Cardiac resuscitation was achieved in all. Ventricular fibrillation was encountered only 1 time and this was converted by 1 counter shock. Ten animals expired in the first 48 hrs. The cause of death in all appeared to be hemorrhage. This cause of death has been eliminated by more careful attention to the neutralization of the heparin dose with protamine, more meticulous care with the surgical hemostasis and careful washing and sterilization of the plastic tubes of the oxygenator apparatus.

The longest period of arrest was 35 min. This heart was resuscitated in the usual manner with resumption of ventricular contractions 50 sec. after the release of the aortic clamp. The pump-oxygenator was discontinued 1 min. later. Parts of the electro-cardiographic record are shown in Figure 2.

A series of 8 children with interventricular septal defects has been operated on using the adjuncts of the pump oxygenator and induced cardiac arrest with acetylcholine. As in the animals it was found that the intracardiac part of the operation was greatly facilitated. The edges of the defects could be visualized accurately although in several instances it was necessary to divide 1 or 2 papillary muscles in order properly to retract a leaflet of the tricuspid valve. The repair was reinforced with a pledget of Ivalon sponge in two cases.

The behavior of the electrocardiogram in a typical case is shown in Figure 3.

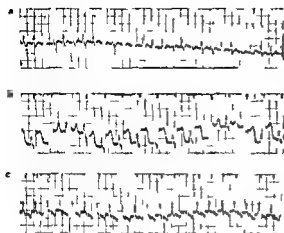
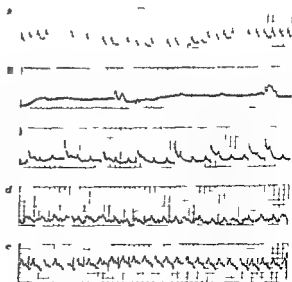


Fig 2 Lead II of the electrocardiogram of a dog, during an experiment in which the heart was arrested for 3 min with acetylcholine a Preoperative tracing b 5 min after the release of the aortic clamp (10 min after the beginning of the arrest) c Tracing taken 12 hrs later

In 6 of the 8 clinical cases of interventricular septal defect a normal sinus rhythm was established following the induced arrest. In the other 2 cases ventricular fibrillation was significant. One patient presented 2 episodes of fibrillation before the cannulation procedures. In fact the subclavian cannula was inserted while the circulation was maintained by manual systole. This heart (a very large one) resumed ventricular fibrillation following the closure of the defect and a regular rhythm was not established. The heart of another patient fibrillated during resuscitation, was controlled by countershock and a good aortic pressure was being produced, but after the patient was beginning to recover consciousness the heart slowed down to stand still and intractable fibrillation occurred after massage. Whether or not the fatal outcome in these 2 cases was related to the induced cardiac arrest or the pathologic conditions encountered and the suturing procedures cannot be stated with certainty. Two of the patients in whom a sinus rhythm was established died in the immediate postoperative period of causes unrelated to the heart itself: one had cerebral edema after improper cannulation of the superior vena cava and another had intrathoracic hemorrhage with inadequate blood replacement.

Fig 3 Lead II of the electrocardiogram of a patient having closure of an interventricular septal defect with the pump oxygenator and acetylcholine induced cardiac arrest. a Preoperative tracing b shows the appearance of the first ventricular contraction 1 min after the removal of the aortic clamp c 2 min after the release d four min after the release e 24 hrs after the operation



SUMMARY

Animal experiments have shown that acetylcholine is a safe and effective cardioplegic agent for the heart in both the hypothermic and normothermic states. In a series of 8 open heart operations for the repair of interventricular septal defects induced cardiac arrest has appeared to be a valuable adjunct.

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METABOLIC CHANGES ASSOCIATED WITH THE USE OF THE MICRO BUBBLE TYPE PUMP OXYGENATOR UNDER NORMOTHERMIC AND HYPOTHERMIC CONDITIONS*

SAM E STEPHENSON, JR, JOHN L SAWYERS, GEORGE L HOLCOMB,
FRANK GOLLAN, ROLLIN A DANIEL, JR AND H WILLIAM SCOTT, JR

In an experimental investigation of the clinical applicability of the combination of hypothermia and extracorporeal circulation numerous physiological, biochemical and metabolic studies have been made. These studies were undertaken in an effort to evaluate the physiological changes brought about by the use of an extracorporeal circuit in survival experiments under either hypothermic or normothermic conditions. This report concerns data collected from a group of 150 animals subjected to cardio-pulmonary bypass with or without ventricular cardiectomy. Perfusion and oxygenation were accomplished by the micro bubble pump oxygenator as described by Clark Gollan and Gupta¹ or 1 of 4 other oxygenators designed by Gollan and based on this principle. It was also hoped that these studies might reveal an explanation of certain deaths encountered in this series in which detailed gross and microscopic pathologic study failed to explain the death of the animal. These unexplained deaths constituted over 50 per cent of the failures in this group of experiments.

For this study blood samples were obtained for biochemical and formal clement analysis preoperatively during perfusion at the completion of the experiment and daily for 3 days postoperatively. These samples were in

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alyzed for serum chloride potassium sodium, total serum protein with albumin globulin ratios, carbon dioxide combining power, non protein nitrogen sugar, calcium phosphate plasma hemoglobin, platelets, packed cell volume and white cell count

METHOD

Unselected mongrel dogs were anesthetized with sodium pentobarbital. Hypothermia, when used, was induced by the immersion technique lowering the esophageal temperature to 32°C. The animal was intubated and attached to a modified Pneophore respirator during the cooling process. A bilateral thoracotomy in the third intercostal space with transection of the sternum was used. The animal was then prepared for cannulation and connection to the pump oxygenator after sodium heparin (5 mg/kg) was given intravenously. Total cardio pulmonary bypass was carried out for a minimum of 30 min and ventricular cardiotomy when used, for a minimum of 15 min. Total body perfusion was accomplished by using the Gollan pyrex or lucite pump oxygenator or the Sigmamotor pump with 1 of the 3 modified Gollan oxygenators*. Body temperature was further lowered to 24 to 28°C in some of the experiments by the use of a cooling coil in the circuit. The pump oxygenator in approximately one half of the hypothermia experiments was primed with mammalian Ringer's solution, no donor blood being necessary until completion of the perfusion.

Postoperatively the heparin was neutralized by protamine sulfate after the method of Gollan, Hamilton and Meneely.² A number of the dogs were rewarmed by the immersion technique and the remainder by slow rewarming to a body temperature of 36°C. Penicillin and streptomycin were administered for 7 days. Small fresh whole blood transfusions were given to maintain a normal packed cell volume. The dogs were allowed to eat and drink as soon as they reacted. Three to 500 cc of 5 per cent dextrose/water was occasionally necessary on the first postoperative day, but in general no other electrolyte replacement was given.

Perfusion flow rates in this series of animals varied rather markedly under hypothermia. The earlier experiments were performed by attempting to pump physiologic cardiac outputs for the body temperature of the dog. These values were calculated after the method of Bigelow.³ In the later experiments under hypothermia and in all the experiments under normal temperatures flows were calculated to be approximately 3 times the 100 g/m^2 factor as determined by Cohn and Lillehei.⁴

RESULTS

The data are tabulated and condensed into Table I which presents an average of many determinations for each study. The preoperative immediate postoperative and first postoperative day averages only are presented for the sake of brevity. These results show the major trends in all studies. With the exception of the total serum protein and variations in the albumin

*The Clark Gollan pump oxygenator utilizes intermittent suction and pressure to circulate the blood as compared to the finger like compression of the blood conduits by the Sigmamotor pump. The suction pressure is controlled by a simple electronic circuit containing 3 electro magnetic relays and energized by the perfusate coming in contact with positive and negative electrodes.

Table 1 Average Values of Biochemical Determinations

DETERMINATIONS	HYPOTHERMIA			NORMOTHERMIA		
	1ST OP	POST OP	1ST POST OP DAY	1ST OP	POST OP	1ST POST OP DAY
Blood Sugar mg %	95	91	80	96	90	82
NIN mg %	30	29	25	26	29	27
Calcium mEq %	99	90	87	109	96	93
Phosphorus mg %	405	31	42	401	44	45
CO ₂ mEq/L	220	174	189	234	162	195
Chloride mEq/L	102	1149	1038	1047	1101	1055
Sodium mEq/L	1447	1455	1402	1453	1466	1450
Potassium mEq/L	34	27	34	40	30	38
Total Serum Protein G %	62	28	48	59	45	47
Albumin G %	32	14	24	35	24	28
Globulin G %	30	14	24	24	21	19

globulin ratio all values returned to the normal average range (± 1 standard deviation) within the first 3 postoperative days

From a study of Table 1 one sees that the blood sugar non protein nitrogen serum calcium and serum phosphate remain fairly constant both under hypothermia and at normal temperatures. A further evidence of physiologic equilibrium is found in the sodium and potassium determinations. There is an over all average increase in the serum sodium of 0.9 mEq/L and a decrease in the serum potassium of exactly the same amount. The highest serum sodium recorded was 157.5 mEq/L and the lowest potassium 1.9 mEq/L. A constant change in the serum chloride level was present however but with no significant difference in regard to body temperature differential. There was a constant elevation of the serum chloride after perfusion averaging 8.1 mEq/L. The variation in chloride ranged from a low of 85.9 mEq/L preoperatively and 99 mEq/L postoperatively to respective highs of 119.4 mEq/L and 123 mEq/L.

The lowering of the carbon dioxide combining power is also constant in our study the average change being a little more severe at normal temperatures (7.2 mEq/L compared to 5.6 mEq/L). The over all average decrease of CO₂ was 6.23 mEq/L. On the average all electrolyte studies are within the range of normal by the first postoperative day.

The average drop in total serum protein with perfusion under hypothermia was 3.4 gm per cent compared with 1.4 gm per cent at normal tempera-

alyzed for serum chloride potassium, sodium, total serum protein with albumin globulin ratios, carbon dioxide combining power non protein nitrogen sugar, calcium, phosphate, plasma hemoglobin platelets packed cell volume and white cell count

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mize changes in the CO_2 content of the blood. The average decrease of 6.23 mEq/l has not seemed of sufficient magnitude to require any corrective measures in the postoperative period.

The most marked variation from normal in the biochemical studies was found in the total serum protein determinations and albumin globulin ratios. This appears at first glance to be more marked with hypothermia. Most of the hypothermic animals, however, were perfused with a pump oxygenator whose priming volume was 300 to 500 cc. In most cases a large per cent of this volume was composed of mammalian Ringer's solution and in many dogs no donor blood was used in the actual perfusion. The average decrease in protein of 3.4 gm per cent with hypothermia is therefore due in part to a simple dilution factor. No definite cause has been found for the four fold loss of albumin over globulin.

The most distressing change is previously observed by Gollan *et al.* - was the marked reduction in white cells and platelets. This drop was more profound with perfusion under hypothermia. The 3 factors responsible for this are (1) the physiologic reduction in white cells and platelets which occurs with hypothermia, (2) the dilution factor brought about by the use of Ringer's solution and (3) the large surface area necessary for defoaming in the micro bubble type pump oxygenator which traps numbers of these cells in the interstices of the nylon filaments. These changes as mentioned before are rapidly reversed by the administration of fresh or decalcified whole blood.

The unexplained deaths in this series of animals remain a mystery. None of the alterations observed in this study can be incriminated as being of sufficient magnitude to account for the death of the animal.

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Table 2 Average Values for Formed Elements of Blood

DETERMINATION	HYPOTHERMIA			NORMOTHERMIA		
	PRE OP	POST OP	1ST POST OP DAY	PRE OP	POST OP	1ST POST-OP DAY
WBC/m ³	8 970	2 222	15 635	13 438	5 553	42 150
Platelets/m ³	230 000	91 000	167 000	210 666	39 739	196 000
Plasma						
Hemoglobin	5 mg%	12.3 mg%	--	5 mg%	33 mg%	--
ICV	38.5 mm	26.4 mm	39 mm	39 mm	32 mm	40 mm

tures. This loss of serum protein was primarily at the expense of the albumin fraction irrespective of whether these animals were subjected to perfusion at normal or lowered body temperatures. The amount of this albumin deficit was frequently great enough to cause a reversal of the albumin globulin ratio. The average loss of total serum protein when the pump oxygenator circuit was primed with whole blood was 1.4 gm per cent, the deficit consisting of a four fold loss of the albumin fraction over the globulin fraction. This change has also been confirmed by electrophoretic patterns. The animal usually corrected this protein deficit in the first postoperative week if a severe infection was not present.

The second table shows the results of measurements of the formed elements of blood and of plasma hemoglobin determinations. It is apparent from this table that changes in the formed elements of the blood are similar under each of the experimental conditions but that they are more marked with hypothermia. The use of fresh whole blood transfusions after completion of the perfusion rapidly reversed these changes and samples of blood drawn 2 hrs. after the completion of the procedure frequently yielded normal values. There was a marked elevation of the white blood cell count on the first postoperative day which gradually returned to normal. Total eosinophil counts in both the hypothermic and normal temperature groups revealed evidence of satisfactory adrenal function in the immediate and late postoperative period.

The average plasma hemoglobin level of 12.3 mg per cent with hypothermia is not statistically different from the level of 33 mg per cent at normal temperatures as the hypothermic group is composed of a smaller number of determinations.

DISCUSSION

In general these studies of the major cations, anions, proteins and formed elements of the blood in animals submitted to total cardio pulmonary bypass with micro bubble pump oxygenators have shown no significant changes which are not rapidly reversible irrespective of whether body temperatures were lowered or maintained at normal levels.

The consistent elevation of serum chloride observed in the early postperfusion period in these experiments may be due in part to the addition of normal saline or Ringer's solution to the perfusate during the pumping procedure.

In this study the gas mixture in the oxygenating chamber was composed of 95 per cent oxygen and 5 per cent carbon dioxide. This seemed to mini-

INCIDENCE OF AIR EMBOLISM

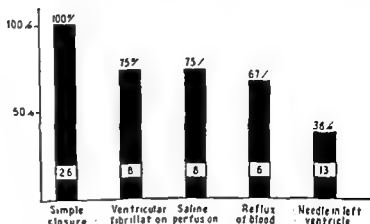


Fig 3 Incidence of air embolization using various techniques of closure. Figures in light blocks indicate number of experiments in each group.

cephalic trunk and a 7 mm T shaped cannula was inserted through a slit in the descending aorta. The cannula in the ascending aorta allowed blood to flow into a glass bubble trap from which it emerged to enter the T cannula in the descending aorta. By ligating the aorta between the 2 brachiocephalic trunks the left ventricular output was shunted through the bubble trap and returned to the descending aorta.

Blood was drawn off by catheters in the venae cavae into the heart lung machine which consisted of a Sigmamotor pump and DeVilbiss type bubble oxygenator. The oxygenated blood was put back into the animal through the T cannula in the descending aorta.

Figure 1 demonstrates the circulatory arrangement during creation and closure of the defect. Figure 2 demonstrates circulation through the bubble trap after closure of the defect and cessation of artificial perfusion.

RESULTS

Atrial septal defects were created and closed 61 times. In no instance was air recovered from the aorta during the period that the heart lung apparatus was maintaining the animals cardiorespiratory function. The incidence of demonstrated air embolism with various techniques of closure is shown graphically in Figure 3.

AVERAGE VOLUME OF AIR EMBOLI

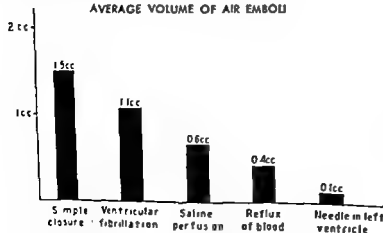


Fig 4 Average volume of emboli associated with various techniques of closure.

AN EXPERIMENTAL METHOD FOR MEASUREMENT OF AIR INTRODUCED INTO THE GREATER CIRCULATION DURING OPEN HEART CLOSURE OF ATRIAL SEPTAL DEFECTS

V L WILLMAN E C NEVILLE AND C R HANLON

The introduction of air into the systemic circulation during open heart closure of septal defects is a serious hazard. The amount of air necessary to cause serious harm has been studied experimentally in dogs by Gloghagan and Lam.¹ The incidence of air embolism associated with various techniques however has not been recorded nor has the quantity of air introduced been determined.

This report describes an experimental method for measuring the amount of air introduced into the greater circulation during open heart closure of atrial septal defects using a heart lung apparatus. The efficacy of various methods designed to avoid air embolism has been compared.

METHOD

Mongrel dogs of 10 to 15 kg weight were anesthetised with thiopental sodium. With the animal supine the chest was entered through a bilateral fourth intercostal space trans sternal incision. The azygos vein was ligated and the cavae encircled with umbilical tapes. A 7 mm straight metal cannula was introduced into the ascending aorta through the right brachio

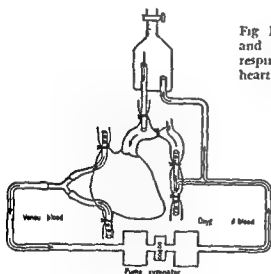
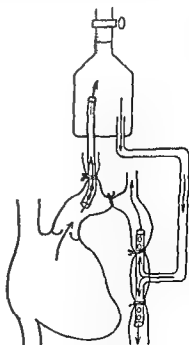


Fig 1 Circulatory arrangement during creation and closure of the atrial septal defects. Cardio respiratory function is being maintained by the heart lung apparatus

Fig 2 Circulation through the bubble trap after closure of the defect



METHOD

Details concerning the Cibbon type mechanical pump-oxygenator and the recording equipment have been given elsewhere.¹ Forty five patients of the group were selected for more particular study on a basis of weight and rate of blood flow obtained during perfusion and an analysis of their records has shown certain related events to occur during and after the period of perfusion.

Body Weight and Blood Flow During Perfusion For the 80 patients in whom these two variables were studied it was found that as the weight of the patient increased the flow in terms of cubic centimeters per kilogram per minute decreased. Since many factors influence the actual flow obtained during perfusion the points were widely scattered but a good correlation was observed when the patients were placed in groups for each 10 kg. of body weight and the average flow for each group was plotted against the average weight (Table 1). Data obtained at preoperative cardiac catheterization showed a similar relationship between body weight and systemic flow in terms of cubic centimeters per kilogram per minute.

Arterial Pressure and Blood Flow During Perfusion In all perfusions the aortic blood pressure varied directly with the rate of perfusion flow and over the comparatively small range of flow (0.8 to 1.0 L./min.) which was available for study the relation appeared to be a linear one. In general the mean aortic blood pressure recorded during perfusion was lower than the values obtained in the preperfusion and postperfusion periods even when the rate of blood flow during perfusion approximated the previously determined cardiac output of the patient. Consistently during and after the period of perfusion the normal relation of the central to the peripheral arterial systolic pressure was disturbed in that the central systolic peak exceeded the peripheral by 5 to 20 mm./Hg.

Central Venous Pressure. Central venous pressure varied directly with the rate of blood flow but the actual pressure about which the changes occurred appeared to be related to the volume of blood within the patient. When venous pressure was plotted against the rate of blood flow several curves

Table 1 Relation of Body Weight to Rate of Perfusion Flow

	WEIGHT OF PATIENTS IN KG.					MORE THAN 50
	LESS THAN 10	10-20	20-30	30-40	40-50	
Cases in group	13	27	15	5	11	14
Weight kg.						
Average	5.3	16.2	24.5	33.3	47.8	59.8
Range	3.7-9.5	11.4-19.1	20.5-30	31.8-34.5	44-49.5	50.9-84.1
Flow cc/kg./min.						
Average	92	55	62	59.8	45	48.8
Range	42-220	4-126	25-104	41-90	20-91	30-88

It can be seen that embolization always occurred when the defect was simply sewed without precautions. Induced ventricular fibrillation flooding of the atrium with saline solution or allowing blood to fill the atrium just prior to final tightening of the suture line lowered the incidence of embolization slightly. Venting of the left ventricle by means of an 18 gauge needle at the apex lowered the incidence of embolization substantially.

Figure 4 compares these various techniques as to average volume of embolus. The size of the embolus is smallest when a needle is maintained as a vent in the apex of the left ventricle during the open heart procedure. This technique is similar to that described by Miller and co-workers.²

SUMMARY

A method is presented for evaluation of systemic air embolization by means of a bubble trap placed at the outflow from the left ventricle. Measurements were made following creation and closure of atrial septal defects using a heart lung apparatus.

Several common techniques designed to prevent air embolism were evaluated. Venting the left ventricle by means of a needle in the apex during closure considerably decreased both the incidence and volume of emboli.

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STUDIES IN EXTRACORPOREAL CIRCULATION

III THE RELATION OF BLOOD FLOW AND BLOOD VOLUME DURING EXTRACORPOREAL CIRCULATION IN MAN*

DAVID E. DONALD AND EMMERSON A. MOHITT

Although a variety of mechanical oxygenators and blood pumps have been described for extracorporeal circulation and oxygenation of blood, two main approaches to the perfusion of the patient have been developed. In one the arterial pump is set to deliver a previously selected and constant amount of oxygenated blood to the patient irrespective of the volume of venous blood delivered per minute from the patient to the apparatus. In the other the rate of return of oxygenated blood from the apparatus to the patient is governed by and is at all times equal to the rate of outflow of venous blood from the patient to the apparatus. It was under this second type of perfusion that the following observations have been made on 80 patients undergoing open intracardiac operations at the Mayo Clinic.

*The Mayo Clinic, Rochester, Minn. and The Mayo Foundation, Rochester, Minnesota (a part of the Graduate School of the University of Minnesota).

of this controlling volume of 50 cc. Thus relatively small changes in the circulating volume of the patient-machine circuit are reflected in significant changes in the rate of perfusion flow. While it has proved impossible to quantitate the relation of the changes in flow to the changes in volume, the knowledge that the withdrawal from the circuit of as little as 30 cc. of blood will produce a measurable change in the rate of flow has proved invaluable in the proper maintenance of the perfusion and support of the patient.

Studies at present in progress in the laboratory indicate that during perfusion in the dog a withdrawal of blood amounting to 10 per cent of the blood volume will reduce the rate of blood flow by almost 50 per cent and that flow will continue at this reduced value until the volume abstracted is returned.

The direct relation of aortic and central venous pressure to flow, the simultaneous response of both these pressure systems to alterations of flow, the apparent inability of the patient to compensate for loss of blood, and the marked sensitivity of the patient-machine rate of flow to alterations in circulating volume have led to the belief that under conditions of extracorporeal circulation the patient behaves as a relatively noncompliant system. If this be so, then it follows that the rate of flow in the system will change with and be sensitive to alterations in volume within the system. It is recognized that this concept cannot be verified in the operating theater but must be studied under more controlled conditions in the laboratory.

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A SIMPLIFIED PUMP OXYGENATOR WITH FLOW EQUAL TO NORMAL CARDIAC OUTPUT*

JEROME HAROLD KAY AND ROBERT A. GAERTNER

At present there are only two practical methods of oxygenating blood: spreading the blood in a thin film in an atmosphere of oxygen or mixing bubbles of oxygen in the blood. Although the bubble oxygenators are efficient, the difficulty of obtaining high flows, the difficulty of complete removal of bubbles, and the destruction of many of the constituents necessary for the clotting of blood are distinct disadvantages. In all of the film oxygenators an important factor is the amount of surface area of blood that must be exposed to the gas phase.

*From The Clinic of Surgery, National Heart Institute, Bethesda, Md.

could be drawn for a single perfusion. The curves were concave toward the pressure axis but the position of each curve on the graph seemed related to the blood volume of the patient since it lay at a higher pressure level when the blood volume was normal than when the blood volume was reduced as after a sudden loss of blood.

A significant but unexplained finding was that when alterations occurred in the outflow from the apparatus to the patient the accompanying changes in aortic and central venous pressure were almost simultaneous in their time of occurrence.

Flow of Blood and Oxygen Saturation of Mixed Venous Blood. Data from 45 patients demonstrated the following points:

1. In the individual patient the oxygen saturation of the mixed blood from the caval veins varied directly with the rate of perfusion flow.

2. When the patients were placed in 2 groups on a basis of body weight (a) in each group the higher oxygen saturation of venous blood was associated with the higher flow and (b) at each level of venous oxygen saturation the group with the higher weight exhibited the lower flow in terms of cubic centimeters per kilogram per minute. These points are illustrated in Table 2 which also indicates the fairly wide range of flow associated with each level of venous oxygen saturation.

Perfusion Flow and Alteration of Blood Volume. Proper to the use of the Gibbon type pump oxygenator is the concept that the patient determines his own rate of flow during the perfusion. The technique of perfusion has been designed to establish conditions which enable the patient to deliver blood flow at a maximal rate to the machine.

The experience gained with these 80 patients has created the impression that (1) alteration of blood volume is the principal factor affecting the rate of blood flow during perfusion and (2) under conditions of extracorporeal circulation the usual mechanisms whereby the patient compensates for loss of blood are severely depressed or in abeyance.

The control system of the Gibbon type pump oxygenator makes this a constant volume machine in which input and output are constantly balanced and the apparatus itself operates through full scale on a controlling volume of 50 cc. Since the volume of blood in the apparatus is constant and the patient is unable to respond in the usual fashion to a loss of blood, a reduction in the circulating volume of blood in the patient is reflected in a reduction

Table 2 Relation of Venous Oxygen Saturation to Perfusion Flow

GROUP	WEIGHT kg	VENOUS O ₂ SATURATION PER CENT			
		51-60	61-70	71-80	81-90
Less than 20 kg		FLOW cc/kg/min			
Average	11.5	56 (1*)	68 (8)	87.5 (8)	130.5 (7)
Range	3.7-19.1		45-111	60-147	72-220
More than 20 kg					
Average	44.1	26.5 (2)	41.5 (8)	53.5 (4)	67.8 (7)
Range	20.9-84.1	25-28	28-63	42-66	54-89

*Figures in parentheses indicate number of patients in each unit.

establish the film the blood was brushed on the screen. Samples were then obtained with this length at the desired flows. The rate of flow was adjusted by changing the height of the bottle of blood. A piece of screen was then cut off the bottom of the strip so that it measured the next shorter length to be tested. Samples were again obtained at the same flows. This procedure was repeated for each succeeding shorter length to be tested. The per cent saturation was plotted against the screen length and a curve was obtained for each flow rate.

Two types of screen were used in these experiments. (1) Saran window screen and (2) stainless steel screen used in the Carbon oxygenator.

RESULTS

In a typical experiment the oxygen capacity was 18 l vol per cent and the saturation of the venous blood was 61.3 per cent. Three flow rates were used: 10, 20, and 30 ml/min. The shape of the curves was similar. The major part of oxygenation occurred over the first 20 cm of screen length. Ten ml/min appeared to be the optimum flow. At this rate and a screen length of 20 cm the saturation was raised from 61.3 per cent to 91.1 per cent. This represented an increase of 30 per cent. With an additional 10 cm of screen the saturation was raised only 1.4 per cent and with a total length of 45 cm of screen the saturation was 99 per cent or only 8 per cent more saturated than the blood at 20 cm (Fig 2). In this particular experiment Tyler #538 Type 304 Stainless Steel Ton Cap Screen was used. However the results were the same whether the stainless steel or plastic screen was used.

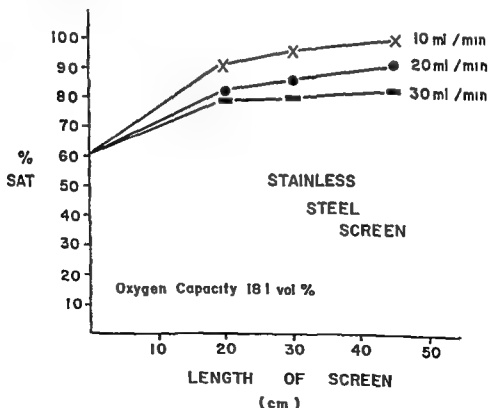


Fig 2 Reveals the % oxygenation with various flow rates and various lengths of screen with a constant screen width (2.5 cm)

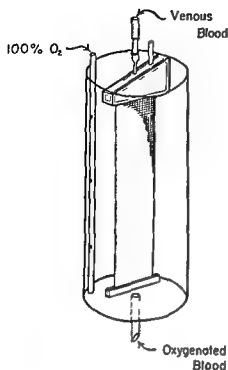


Fig 1 Apparatus used to determine the optimal screen length and rate of flow for a stationary screen oxygenator

After first determining the optimum length of screen and optimum blood flow on the screens a pump oxygenator of the Gibbon type was constructed and used successfully in dogs

METHOD

In order to determine the effect of screen length on the efficiency of the stationary screen oxygenator the following experiment was performed. A single test apparatus was used for all the experiments (Fig 1). This consisted of a small plastic distributing chamber with an inlet for venous blood and an outlet for the removal of air. From this distributing chamber a strip of screen 2.5 cm wide was suspended. In order to add weight to the screen and thereby keep it straight 2 strips of plastic were clamped at its lower edge.

The distributing chamber and attached screen were suspended in a plastic cylinder. Ten liters per minute of 100 per cent oxygen were blown into this cylinder through a glass tube with 3 openings. The openings were directed toward the wall of the cylinder instead of toward the film of blood so that there would be little or no turbulence produced in the blood phase by the jets of gas.

Human blood of the same type was pooled for the experiments. In order to simulate the conditions seen clinically the saturation of the venous blood was raised to between 35 and 60 per cent by swirling it in a flask in an atmosphere of 100 per cent oxygen. Samples of oxygenated blood were collected in a small beaker under oil. Oxygen contents were measured by the technique of Van Slyke and McNair. Carbon dioxide contents were not determined.

For each experiment a strip of screen was attached to the distributing chamber. This strip measured the greatest length to be tested in that given experiment. The pooled venous blood of 35 to 60 per cent saturation was run into the distributing chamber with an intravenous drip set. In order to

mal as well as the quantity of blood in the bottom of the oxygenator can be regulated by merely changing the speed of the arterial pump. During the perfusion period only the rate of the arterial pump is changed and by maintaining a constant level in the bottom of the oxygenator the entire extracorporeal system is kept balanced. The coronary sinus blood is aspirated by gentle suction (10 cm of water) into a reservoir (CR) and then flows by gravity into the venous reservoir.

Using this pump oxygenator in 10 consecutive animals total cardiac bypass was carried out for 35 to 15 min. In all of the animals the right ventricle was open for 30 min and 8 of these animals were chronic survivors.

SUMMARY

A very efficient, simply designed and constructed pump oxygenator of the Gibbon type is described. This apparatus is capable of oxygenating and pumping 5 L of blood per minute.

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THE ULTIMATE IN VIVO SURVIVAL OF ERYTHROCYTES WHICH HAVE CIRCULATED THROUGH A PUMP OXYGENATOR*

WILMER C. HEWITT, JR., IVAN W. BROWN, JR., G. S. EADIE, WIRT W. SMITH, AND W. C. SEALY

The recent increasing use of pump oxygenators in open cardiac surgery has resulted in much interest in the effect of such devices on the blood exposed to them. Up to the present interest has focused mainly on their effect on the blood coagulation mechanism and also on immediate red cell hemolysis which with the present pump oxygenators it is agreed does not occur to any great extent. Few if any studies have been carried out on the long term survival of red cells which have been circulated through an extracorporeal circuit. Evidence is now accumulating which points to deleterious effects arising at interfaces between blood and foreign surfaces or blood and oxygen bubbles. The following is a preliminary report of studies of *in vivo* erythrocyte survival as affected by pump-oxygenators.

METHOD

The oxygenator employed in these studies has been described previously.¹ Briefly it consists of a plastic bag containing 650 cc of pure oxygen with additional capacity to accommodate 500 cc of blood. As the venous blood enters from the patient the bag oxygenator is gently rocked to and fro by

*From the Departments of Surgery and of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina. Supported in part by research grants from the National Heart Institute, USI HS 11782 and H 1226 (C5).

DISCUSSION

Apparatus With the information from the above experiments an oxygenator was designed and constructed. It is similar to that described by Gibbon except for the screens being shorter and wider than those of Gibbon. The optimal length for oxygenation is approximately 25 cm and the optimal width for convenience is 10 cm. If the screens are longer the extra length is wasted and this results in greater hold up of the blood and decreased efficiency of oxygenation.

Since the maximum blood flow per 25 cm of screen width is around 20 ml/min in order to construct the oxygenator to adequately oxygenate 5 L of blood we used 610 cm of screen width. This surface area was obtained by using 16 screens 40 cm in width and 25 cm in length.

Only 2 pumps are required for the apparatus. Those used are of the DeBakey non occlusive roller type. Each pump compresses a single piece of Tygon tubing 0.5 inch in diameter. The output of each pump is directly proportional to the revolutions per minute and ranges from 0 to 10,000 ml/min.

The circuit is illustrated in Figure 3. One of the pumps is used to maintain a constant film on the screens and is in the internal circuit. This internal circuit consists of the venous reservoir (VR), internal circuit pump (ICP), oxygenator (O), and the low resistance shunt from the bottom of the oxygenator to the bottom of the venous reservoir. The internal circuit pump is set so that the flow is greater than the amount siphoned from the arterial or returned to the arterial. As first described by Gibbon, the internal circuit pump maintains a constant film of blood on the screens. The quantity of blood on the screens therefore does not vary. Venous blood is siphoned from the side and therefore any changes in venous return are automatically adjusted for by an immediate similar opposite change in the shunt from the bottom of the reservoir to the venous reservoir. The arterial pump (AP) returns oxygenated blood from the bottom of the oxygenator through a filter (F) to the artery. During total cardiac bypass the blood pressure of the ar-

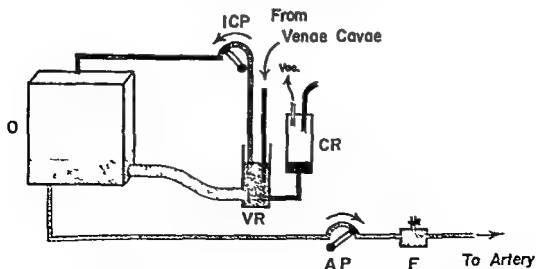
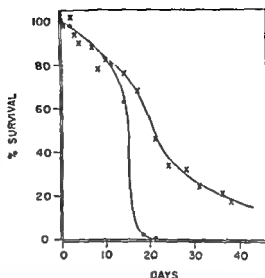


Fig. 3. Schematic diagram of pump oxygenator (See text).

Fig. 2 *In vivo* survival of red cells in normal recipients after passage through the pump oxygenator and patient's circulation during 2 human operations for open cardiac surgery lasting 24 min (x) and 2½ hrs (•) Tagging with Cr^{51} . Points corrected for radioactivity decay but not for elution of the isotope



was tagged with Cr^{51} but also when it was tagged with Fe^{59} . In addition the final blood returned to the oxygenator at the close of 2 open cardiac operations on humans was tagged with Cr^{51} and followed in healthy compatible recipients. In both these cases there was a premature loss of the transfused erythrocytes the survival curves showing the same S shaped drop (Fig. 2).

DISCUSSION

The disappearance curve of erythrocytes which have circulated through a pump-oxygenator and have been subsequently transfused into a normal compatible subject frequently shows after a short normal period of loss a sharp S shaped drop sometimes great enough as to indicate total disappearance of transfused cells within 30 or 40 days after transfusion instead of the normal 3 or 4 mo. Whether this occurs or not appears to depend in part on the recipient for the same blood transfused into two recipients may show the loss in one and not in the other. It is of course never seen with undamaged cells.

The significance of this phenomenon is still far from clear. The S shape of the curve seems to mean that the damage has been inflicted on all cells simultaneously and the length of time elapsing before the disappearance of the cells is apparently subject to the usual biological variation—a normal distribution about a mean. The delay before the effect of the damage is seen may possibly be explained in one of several ways. First is the possibility of an immune reaction. Here we must assume that the trauma inflicted by the pump-oxygenator is such as to render the cells antigenic although the undamaged cells are not. After a certain period—1 wk or more—antibody production has reached a level sufficient to cause removal of the affected cells. It must also be assumed that in many cases only a fraction of the cells are traumatized to a degree that would render them antigenic and the removal of cells is therefore incomplete. Such a theory can easily explain the delay but in this case the disappearance curve should be exponential rather than S shaped. Another possibility is that the process causing disappearance of old cells (senescence) is accelerated in some way. It is difficult to see how this could lead to an S shaped curve for it would be necessary to assume e.g. that the acceleration of senescence was less marked in the younger cells as

hand so as to spread the blood over the bag's internal surface. In this way 500 cc of blood can be fully oxygenated within $1\frac{1}{2}$ and 2 minutes without foaming or frothing and with a minimum of trauma. Once oxygenated the blood is pumped back into the patient's subclavian artery directly from the bag while the venous blood is entering another bag and being oxygenated.

The oxygenator is used with the dual Sigmamotor pump model T 65. Except for the silicone rubber pump head tubes, the entire extracorporeal circuit is composed of non-wettable disposable polyvinyl chloride plastic which had been sterilized by autoclaving.

Whole venous blood was drawn from healthy donors into plastic bags containing 15 mg of heparin for each 500 cc of blood. In every case the blood was put through the pump oxygenator and transfused within an hour or 2 after withdrawal from the donor. Except in the human clinical cases a sample of unpumped, unoxygenated blood was tagged and transfused into a healthy compatible recipient as a control. Blood grouping and compatibility tests, including indirect antiglobulin tests, were carried out in all instances on the blood of the donor and that of all recipients. Similarly, in dog experiments animals were first grouped according to the ABCDE classification of Young *et al*² and only compatible recipients chosen on the basis of cross matching tests. In both human and animal experiments sera of all recipients were retested at the end of the study for any demonstrable red cell antibodies which might have been provoked by the red cells transfused.

In vivo red cell survival was followed by tagging the cells with Cr^{51} according to the method of Read.³ The methods of counting radioactivity et cetera and plotting of data have been described previously.⁴ Canine red cells were followed after tagging with both Cr^{51} and Fe^{59} . In these experiments the latter isotope was counted in a well type scintillation counter.

RESULTS

Experiment 1 Fresh heparinized blood from a healthy donor was recirculated through the pump oxygenator at a rate of 450 cc/min. Samples were taken before and after pumping. Each sample was divided into 2 equal aliquots tagged with radiochromium and each aliquot transfused into healthy compatible recipients. In about half of these (6 out of 11) there was an S shaped drop in the survival curve occurring usually in 1 to 4 wks after transfusion (Fig 1). Similar results were seen in dogs not only when blood

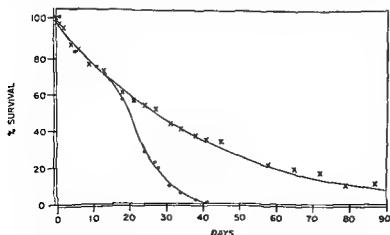
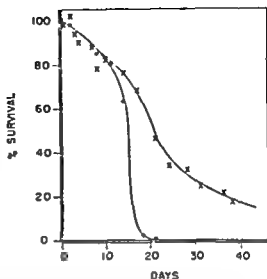


Fig 1 Comparison of *in vivo* survival of red cells in normal recipients before (x) and after (o) passage through the pump oxygenator for 30 min. Tagging with Cr^{51} . Points corrected for radioactive decay but not for elution of the isotope.

Fig. 2. In vivo survival of red cells in normal recipients after passage through the pump-oxygenator and patient's circulation during 2 human operations for open cardiac surgery lasting 24 min (X) and 2½ hrs (•). Tagging with Cr^{51} . Points corrected for radioactivity decay but not for elution of the isotope.



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DISCUSSION

The disappearance curve of erythrocytes which have circulated through a pump oxygenator and have been subsequently transfused into a normal compatible subject frequently shows after a short normal period of loss a sharp S shaped drop sometimes great enough as to indicate total disappearance of transfused cells within 30 or 40 days after transfusion instead of the normal 3 or 4 mo. Whether this occurs or not appears to depend in part on the recipient for the same blood transfused into two recipients may show the loss in one and not in the other. It is of course never seen with undamaged cells.

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well as those which had lived half the potential life span. Such an *ad hoc* hypothesis is far from convincing. More plausible perhaps is the suggestion that the trauma causes damage to the surface of the erythrocyte so that an enzyme system or systems is damaged in such manner that concentration of an essential metabolite within the cell slowly falls to a critical level.

Since in the first experiment cells were labelled with Cr^{51} the question arose whether the presence of this foreign metal played a role or even whether the whole phenomenon were not due to an increased rate of chromium elution rather than cell disappearance. The fact that the same phenomenon is seen when cells are labelled with Fe^{59} built into their hemoglobin seems to be sufficient evidence to refute such a suggestion.

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DESCRIPTION AND EVALUATION OF A ROTATING DISC TYPE RESERVOIR OXYGENATOR*

FREDERICK S CROSS, ROBERT M BERNF, YOICHI HIROSE,
RICHARD D JONES AND EARLE B KAY

It is the purpose of the present report to describe a rotating disc type reservoir-oxygenator which has been thoroughly evaluated in the laboratory and used in 30 clinical cases to date. The essential components of this apparatus are a model TS Sigmamotor pump and a rotating disc type reservoir oxygenator. The basic mechanism of the oxygenator is a series of 59 teflon coated stainless steel discs 0.4 mm thick and 12.2 cm in diameter, mounted 4.5 mm apart by means of stainless steel spacers on a central shaft. The disc assembly is supported horizontally within a silicone coated pyrex cylinder 33 cm long and 13.3 cm in diameter by gasketed end plates of stainless steel likewise treated with silicone resin (Fig 1). Blood introduced at one end passes along the cylinder and is removed from the bottom of the opposite end. The rotation of the discs effectively prevents channeling of blood along the bottom of the cylinder. The oxygenator is primed with 1400 cc of whole blood which immerses the discs 4.1 cm. This gives a thin film of blood of similar width on the surface of the discs to be exposed to oxygen as the discs

rotate. With the oxygenator primed to 1100 cc the static exposed area of the 59 discs is 0.9 sq m. At the generally used disc rotation rate of 120 RPM this gives an exposure area of 108 sq m/min. The discs are rotated by a pulley mounted at one end of the shaft by means of a V belt and a $\frac{1}{80}$ HP motor. Oxygen and 11½ per cent carbon dioxide are supplied through a perforated stainless steel tube mounted within the cylinder above the discs. The oxygen mixture is warmed by passing through tubing immersed in a sand bath heated by an electric mantle. To prevent condensation within it the oxygenator is warmed by a wrapping of silicone rubber covered resistance wire the temperature of which is controlled by a variator. All electrical connections are mounted on a platform over 5 ft. above the floor to eliminate explosion hazards. Provision is made at the inlet face of the oxygenator for filling prior to use, the addition of blood to the cylinder during a run, and for sampling the venous return to the oxygenator from the patient. The output face is provided with openings to vent off the circulating oxygen and carbon dioxide for the collection of samples of oxygenated blood and to receive a thermo couple for the continuous monitoring of blood temperatures. Coronary sinus blood has been collected by means of a sump type suction tip and pumped into a decompressing chamber at the inlet face of the oxygenator from which it is returned to the oxygenator. No bubble traps or filters have been placed in the arterial line.

The oxygenator can be disassembled easily into its component parts for cleaning. It is sterilized in the autoclave after reassembly including the oxygen flow tube and the heating wire. The inside is then dried by flushing with oxygen or air while still hot, or by standing in the hot autoclave. The connecting tubes are sterilized in zephiran for 24 hrs. and then rinsed and mounted in the pump circuit. The tubing is then rinsed with at least 2 L. of saline solution before being connected to the patient.

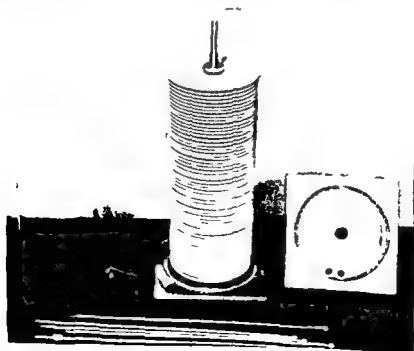


Fig 1 Reservoir oxygenator disassembled to show component parts

METHOD

Effective pump oxygenators must maintain circulation as well as oxygenation during total cardiac bypass with a low morbidity and mortality rate. To determine these functions of the present pump oxygenator both acute and survival experiments on animals were carried out as well as studies on cow blood from a local slaughter house. Subsequently complete physiologic studies were carried out on the 30 clinical cases done to date. These studies included accurate pre and postoperative weights, continuous mean arterial blood pressures, blood studies for oxygen and carbon dioxide content, pH, plasma hemoglobin, hematocrit, platelet and cell counts, total proteins and A/G ratio, as well as electrolyte studies. The cow blood studies were used primarily to evaluate the oxygenating capacity and efficiency of the oxygenator.

RESULTS

Mortality and morbidity were necessarily high in the developmental stages of the pump oxygenator. However, after the basic design and techniques were standardized, a series of 20 dogs was obtained in which there were 2 deaths. In 11 of the dogs, simple cardiac bypass without cardiectomy was done with 1 death due to undetermined causes, and in the remaining 9 dogs, a ventricular cardiectomy was done with 1 death due to postoperative ventricular fibrillation following the institution of a citrated blood transfusion. There have been no deaths attributable to the oxygenator *per se* in the first 30 human cases operated upon.

Average mean arterial blood pressure obtained in 11 bypass experiments without cardiectomy ranged from 50 to 78 mm Hg. About the same values were obtained in humans at flow rates from 40 to 115 cc/kg/min. Patients were carried with mean arterial blood pressures as low as 43 for periods up to 32 min without apparent ill effect. In perfusing both animals and humans it was frequently necessary to add neosynephrine or Vasoxyl to the oxygenator blood to prevent an initial blood pressure drop which frequently accompanied the start of the perfusion. Attempts at maintaining high arterial pressures by increasing perfusion rates alone were not uniformly successful.

A specific value as to the oxygenating capacity and efficiency of the present oxygenator has not been obtained. With such factors as oxygen flow rates, disc rate, and blood temperature held constant, the efficiency of the oxygenator varied not only with the blood flow rates but also with the degree of desaturation of the venous blood returning to the oxygenator. With low oxygen saturations in the venous blood the oxygenator was more efficient in that more cubic centimeters of oxygen were added to the blood per minute, but lower arterial oxygen saturations were obtained. The reverse was true with higher levels of venous oxygen saturation (Table 1). In the cow blood studies the efficiency of the oxygenator ranged from 67 cc to 207 cc of oxygen per minute added to the circulating blood. The arteriovenous differences ranged from 37 to 13 vol per cent depending upon the circumstances of the perfusion. In humans arterial oxygen saturations ranged from 95 to 103 per cent except in 2 cases which were 92 and 93 per cent. The venous oxygen saturation ranged from 33 to 58 per cent. Table 2 illustrates a typical dog experiment.

Table 1 Oxygenation Experiments Using Cow Blood

EXP NO	BLOOD FLOW cc/min	OXYGEN SATURATION VOL %		A-V DIFF	cc O ₂ /min ADDED TO BLOOD
		ARTERIAL	VENOUS		
2	910	18.9	11.8	7.1	67
9	1350	17.5	11.7	5.8	78
17	2120	15.1	11.1	3.7	99
23	2860	15.8	11.8	4.0	114
21	3080	11.8	5.1	6.7	207
25	3090	16.1	11.8	13	152

Table 2 Typical Dog Bypass Experiment

WEIGHT	BLOOD FLOW cc/min	DISC RATE RPM	O ₂ SATURATION VOL %		cc O ₂ /min ADDED TO BLOOD
			ARTERIAL	VENOUS	
74	1000	120	18.5	10.3	80
	1600		18.9	10.1	131
	2000		18.6	11.8	156

In the animal experiments hemolysis was not excessive. The increase in serum hemoglobin over a 30 min pumping period ranged from 10 mg per cent to 160 mg per cent. Variations in serum hemoglobin could not be related to such factors as disc speed up to 120 rpm or to pump speeds but hemolysis did increase with time. At disc rates in excess of 130 rpm hemolysis was excessive and forming in the oxygenator was present. The average increase in plasma hemoglobin in the human cases during perfusion periods ranging from 12 to 103 min was 53.5 mg per cent. The highest figure obtained was a plasma hemoglobin of 320 mg per cent most of which came from the sump suction.

In neither the animal experiments nor human cases was there excessive destruction of platelets or other formed elements in the blood. In 13 animal experiments the platelets dropped from an average figure of 184,770 to 96,000 or roughly one half of their pre-perfusion value. In humans the platelet counts remained above 120,000 in all but 4 instances. In 10 clinical cases in which they were measured there was no significant change in the plasma fibrinogen or in clot lysis. When protamine sulfate has been given in amounts equal to half the calculated dose of heparin and fresh citrated blood given postoperatively excessive bleeding has not been a problem, especially in the human cases.

In the clinical cases done to date the pH of the arterial blood during the bypass period has ranged from 7.14 to 7.62, the majority being between 7.3 to 7.4. There has been no significant change in the carbon dioxide content during or after a run, when 1½ per cent carbon dioxide is added to the oxygen mixture in the oxygenator. A slight metabolic acidosis existed during the perfusion period which quickly returned to normal spontaneously post-perfusion. All blood studies in the patients have continued to be entirely normal during the postoperative period.

Blood balance during the operative procedure in the human cases was well maintained in that all but 3 of the 30 postoperative weights were within

$\frac{1}{4}$ pound of the preoperative weights. This has been corroborated by normal postoperative hemoglobin and hematocrit values.

DISCUSSION

The pump oxygenator described is simple in design, easy to operate and can be constructed to oxygenate over a wide range of blood flow rates without requiring excessive blood for priming purposes. Although it resembles the Bjork¹ apparatus, it differs in its general design, the use of plastic discs and in its greater oxygenating capacity.

Although the oxygenating capacity of the present apparatus has not been accurately ascertained, it probably has an upper limit of about 2000 cc/min. A larger cylinder with a greater oxygenating capacity has been constructed for use in larger individuals and has been used clinically. A smaller cylinder is being constructed for use in infants in order to reduce the amount of blood needed to prime the oxygenator. The model used in the work to the present time is considered to be the basic experimental unit and continued modifications are being made along lines retaining the basic principles described.

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PREVENTION OF VENTRICULAR FIBRILLATION IN THE NORMOTHERMIC AND HYPOTHERMIC HEART IN THE ADVANTAGE OF QUINIDINE OVER ATRIOCAVAT BLOCK*

SAM E. STEINSON, JR. AND I. BRACHLEY MAIN

The control of ventricular arrhythmia has assumed a role of major importance in a large number of surgical procedures. At the present time ventricular fibrillation must be controlled or prevented for the successful outcome of procedures in three main surgical enterprises, each of which is of major importance. The adequate control or prevention of ventricular fibrillation could bring about the following changes:

- (1) The prolongation of the physiologic safe period for total inflow and outflow cardiac stasis in conjunction with hypothermia.
- (2) The clinical acceptance of the combined use of hypothermia and extracorporeal circulation for prolonged open cardiac procedures thereby utilizing the safety factors derived from each procedure.
- (3) A direct surgical approach on the coronary arteries for relief of arteriosclerotic occlusive disease.

The various reported adjuncts to reduce cardiac arrhythmia were tried by us and failed to yield as satisfying results as have been reported.

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Hyperventilation (Swan) increased CO_2 in respirator air (Nara & Lewis) and atrio caval block with Procaine (Schumacher *et al*) yielded some reduction in the incidence of ventricular fibrillation but left something to be desired.

In the light of previous work by one of us, our experience was extended to hypothermia and a direct comparison of quinidine and atrio caval block was made.

METHOD

The preparation was essentially that of Bailey, La Due and York.¹ Unsclerect mongrel dogs weighing from 5 to 12 kg were anesthetized with 20 to 30 mg of pentobarbital sodium per kilogram of body weight. Intermittent positive pressure or Pneophore type respirators were attached to the animals and a left anterior lateral thoracotomy was performed through the third intercostal space. An 0.5×0.5 cm opening was made in the pericardium and the anterior descending branch of the left coronary artery was isolated at its origin from the left common coronary artery. A ligature of #2 braided silk was passed under the artery for subsequent occlusion. Constant recording electrocardiograms were taken on most dogs. Special care was taken not to injure the accompanying veins and those dogs with injured veins were excluded from the series (>0.1%). At the proper time occlusion of the artery was accomplished by traction mediated through a rubber band sufficient to obliterate the lumen of the vessel but not to displace the heart. Care was taken not to disturb the heart during occlusion or immediately after release.

Drugs were administered through a cannula in the femoral vein starting 10 min prior to occlusion and extending through the occlusive period. Hypothermia when used was accomplished through the immersion technique with temperature taken to $30\text{--}32^\circ\text{C}$ on removal from the ice bath. This yielded a maximum reduction of $26\text{--}28^\circ\text{C}$. When atrio caval block was used it was necessary to modify the procedure to include a bilateral thoracotomy and transection of sternum. A concomitant group of controls proved that this variation in incision had no effect on the incidence of arrhythmia or mortality in the acute experiment.

RESULTS AND DISCUSSION

The early basis of the work to be presented was determined some years ago working in conjunction with the medical department of our hospital.² The findings of that investigation were based on the experimental preparation of 499 animals to first determine the incidence of ventricular fibrillation on temporary occlusion of a major coronary artery and the incidence of fibrillation on release of the temporarily occluded artery and secondly to provide a two fold method to test the efficacy of various anti fibrillatory drugs or procedures. This study subsequently was extended to include 661 animals and to evaluate 12 drugs (Table 1).

Although a number of drugs gave statistically significant protection during the period of occlusion or release quinidine and to a lesser extent procaine gave a highly significant dual protection. This protection of quinidine is significant even in the range of 15 mg/kg which is probably lower than the optimal dose. Time so far has not permitted an evaluation of increasing amounts of quinidine at normal temperatures. Numerous control experi-

Table 1. Ventricular Fibrillation—Normothermic Drug Evaluation

DRUG	NO. OF ANIMALS	FIBRIL- LATED DURING OCCLUSION	FIBRIL- DECEASE DURING OCCLUSION	SUR- VIVORS	FIBRIL- LATED ON RELEASE	FIBRIL- DECEASE AFTER RELEASE	SIGNIFICANCE DURING	SIGNIFICANCE AFTER
Control	330	91	28%	239	169	71%		
Lidocaine 16 mg/kg	19	0	0%	19	14	74%	high	
Propofol 4.6 mg/kg	70	8	11%	62	36	58%	high	suggestive
Quinidine 15 mg/kg	11	3	27%	38	18	47%	high	significant
Xylocaine 16 mg/kg	15	5	33%	10	5	50%		suggestive
C 1191 3 mg/kg	16	3	19%	13	10	77%		signif- icant
Endomide 5 mg/kg	31	4	13%	27	11	52%	high	suggestive
Pravastatin 10 mg/kg	16	3	19%	12	9	69%		signif- icant
Laveril 10, 5 mg/kg	30	15	50%	15	9	60%		
All 5 mg/kg	14	6	43%	8	4	50%		
Regitine 0.5 mg/kg	29	10	34%	19	7	37%		significant
Apresoline 0.5 mg/kg	30	5	17%	25	11	44%	high	significant
Isosoline 3 mg/kg	13	10	77%	3	1	33%		

ments were interspersed among the drug groups to give an added check on the technique.

It is interesting to note some features of this earlier work which makes this a reliable procedure to test and compare various drugs.

1. The incidence of fibrillation on occlusion and on release of the occluded artery varied less than 2 per cent between the first 25 control preparations and the total 330 control animals.

2. The early work extended over a 5 yr period and included some 15 operators. The control group varies less than 1 per cent among these different individuals. We, therefore, believe this to be a stable reproducible preparation.

3. All animals have been autopsied. In only 1 instance has thrombosis been encountered. The difficulty of continued occlusion therefore does not become a problem unless intimal damage occurs.

Table 2 Ventricular Fibrillation—Quinidine vs Atrio caval Block

GROUP		NO. OF ANIMALS	FIBRILLATED DURING OCCLUSION		SURVIVORS	FIBRILLATED ON RELEASE	
			OCCLUSION	DURING OCCLUSION		ON RELEASE	ON RELEASE
NORMOTHERMIC	Control	330	91	28	239	169	71%
	Atrio caval Block	10	1	10%	6	5	67%
	Quinidine 15 mg/kg	11	3	7%	8	18	47%
HYPOTHERMIC	Control	10	1	10%	6	6	100%
	Atrio caval Block	10	6	60	1	1	75%
	Quinidine 30 mg/kg	10	0	0%	10	0	0%

The control of ventricular fibrillation under hypothermia is much more difficult its incidence being reported in various series of experiments from 5 per cent to 75 per cent of preparations. The incidence of fibrillation increases markedly with further lowering of body temperature.

The present work consisted of studying 1 new group of animals numbering 10 dogs each (Table 2). First atrio caval block with xylocaine was compared with the control group and with quinidine at normothermic conditions. This failed to demonstrate any perceptible protection from atrio caval block either during occlusion or on release.

A group of control experiments under hypothermia revealed that all animals developed arrhythmia either during occlusion or on release. When atrio caval block and quinidine were compared under hypothermic conditions the dual protective effect of quinidine was again far superior to the other procedure. In this admittedly small series there has not been an episode of ventricular fibrillation when the dog has received quinidine in doses of 30 mg/kg of body weight.

We believe that both atrio caval block with xylocaine and quinidine tends to slow conduction and lengthen the refractory period of the heart thus rendering the heart less susceptible to fibrillatory stimuli whether they be hypothermic, mechanical, anoxic or metabolic in nature. In combating all these stimuli quinidine in our hands has been superior to all other drugs or procedures tested.

This work has been extended to the 3 previously mentioned fields of endeavor all experimentally and some clinically. The administration of quinidine intravenously to animals extends for 3 to 4 min. the maximum length of time that total inflow outflow stasis can be returned under hypothermia without development of ventricular arrhythmia.

An additional beneficial effect has been observed by us using quinidine in conjunction with hypothermia in the experimental animal. In a consecutive group of 16 animals under hypothermia there was a 57 per cent incidence of fibrillation and a 19 per cent resuscitation rate while in a comparable group

Table 1 Ventricular Fibrillation—Normothermic Drug Evaluation

DRUG	NO OF ANIMALS	FIBRIL LATED DURING OCCLUSION	INCL DENCE DURING OCCLUSION	SUR VIVORS	FIBRIL LATED ON RELEASE	INCL DENCE AFTER RELEASE	SIGNIFICANCE DURING	SIGNIFICANCE AFTER
Control	330	91	28%	239	169	71%	-	
Isocaraine 4.6 mg/kg	19	0	0%	19	14	74%	high	---
Isonestyl 4.6 mg/kg	70	8	11%	62	36	58%	high	suggestive
Quinidine 1.5 mg/kg	11	3	7%	38	18	47%	high	significant
Xylocaine 4.6 mg/kg	10	5	33%	10	5	50%		suggestive
C 1194 3 mg/kg	16	3	19%	13	10	77%	signif icant	
Isendimide 3 mg/kg	31	1	3%	27	11	52%	high	suggestive
Fraserline 10 mg/kg	16	3	19%	12	9	69%	signif icant	
Isoveril P ₂ O ₄ 5 mg/kg	30	10	50%	15	9	60%		
ATI 5 mg/kg	14	6	43%	8	4	50%		
Regitine 0.5 mg/kg	29	10	34%	19	7	37%		significant
Apresoline 0.5 mg/kg	30	5	17%	25	11	44%	high	significant
Priscoline 3 mg/kg	15	10	77%	3	1	33%	-	

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THE EFFECT OF ATRIOVENTRICULAR NODAL BLOCKADE ON FIBRILLATION PRODUCED BY ELECTRIC SHOCK*

WATTS R WEBB

Many factors are known to influence the development of ventricular fibrillation in the mammalian heart. These include various drugs such as the sympathomimetic and parasympathomimetic agents; electrolytes especially the ratio of calcium and potassium; hormones; temperature; the status of the sino auricular node; and the oxygenation and perfusion of the myocardium. The present study has observed the effect of anaesthetization of the atrioventricular node on ventricular fibrillation produced by faradic stimulation. The role of the atrioventricular node and the Purkinje system in ventricular fibrillation has been little studied though Scherf, Shaffer and Blumenfeld¹ have suggested that ventricular fibrillation is of multifocal origin with innumerable sustaining centers many of which may be located in the Purkinje fibers.

METHOD

Healthy mongrel dogs of both sexes weighing between 9 and 15 kg. were anesthetized with pentobarbital. Respiration was maintained by an intermittent positive pump respirator through an endotracheal tube. The right chest was entered through the fourth interspace and the pericardium widely opened anterior to the phrenic. The atrioventricular node is located approximately 1 cm. cephalad to the coronary sinus orifice along the base of the posterior leaflet of the tricuspid valve at the junction of the atrial and ventricular septa. The ventricular septum at this point was probed by a small gauge needle inserted through the right atrial wall. When the A V node or the conduction bundle is stimulated arrhythmias are produced and at this point approximately 1/2 cc. of 1 per cent procaine was injected. If a satisfactory block was not produced other injections were made but usually the procaine was limited to a total of 5 cc.

In 8 dogs the sino auricular node which is located at the junction of the right auricle with the superior vena cava was blocked with procaine.

A monitoring EKG was recorded to verify the nature of the rhythm produced. The ventricles were stimulated with a 180 volt alternating 60 cycle

*From the Department of Surgery, University of Mississippi Medical Center. Supported by National Heart Institute Grant No. H 2806.

of a animal receiving quinidine there was an 18 per cent incidence of fibrillation and a 100 per cent resuscitation rate. In the same group the quinidine did not have the adverse effects as noted in resuscitation under normal conditions.

A very little difficulty has been encountered in the toxic effects as to quinidine experimentally or clinically in these low dosage experiments. The EKG on some animals receiving quinidine will show an occasional ventricular premature contraction. This has by ever been of no consequence.

Our only toxic manifestation clinically was seen in a 2-year old white male who was to undergo a direct approach to the pulmonary valve under hypothermia. Six min after the injection of quinidine (5 mg/kg) he developed an idioventricular rhythm. This has ever returned to a normal sinus rhythm after a 25 min. delay. He subsequently tolerated infusions of a 12 min duration in the correction of pulmonary valvular stenosis without incident. Some minutes following release, however he developed cardiac arrest but was successfully resuscitated. He expired 6 hrs. following surgery after he had been awake and shown no evidence of neurological damage. Post mortem examination revealed other multiple congenital cardiac abnormalities.

The addition of quinidine (5-10 mg/kg) to the perfusate in the experimental use of the combination of extracorporeal circulation and hypothermia has reduced the incidence of fibrillation in prolonged cardiopulmonary bypass from 40 per cent in the early work of Gollan *et al.* to approximately 10 per cent at present. Various consecutive series of experiments including up to 25 animals have been done without a single episode of ventricular fibrillation developing. It has also been possible after administering quinidine as reported by Gollan to run dogs in 0°C. and produce cardiac arrest and recover them rewarming without the development of ventricular fibrillation. The work has been done without hyperventilation or changing the content of carbon dioxide in the inspired air.

Experiments are now in progress to evaluate the protective action of quinidine in the direct surgical approach to the coronary arteries. The work at present is very promising and will constitute a future report.

CONCLUSIONS AND DISCUSSION

1. A method is presented which affords a dual test of anti-fibrillatory drugs and procedures based on 30 control normothermic animals.

2. Under normal temperature low doses of quinidine give the most highly significant dual protective effect. Areas dose also show promise but need still further investigation.

3. Dogs cooled with quinidine under normothermia or hypothermia conditions does not successfully alter the development of ventricular fibrillation from the control series under the conditions of this experiment.

4. Quinidine offers a very highly significant dual protective effect against ventricular fibrillation under hypothermia as tested by ten series of primary coronary occlusion.

5. The use of quinidine experimentally and clinically has offered a marked reduction in ventricular arrhythmias under hypothermia as well as combination with extracorporeal circulation and has significantly increased the percentage of successful resuscitations.

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METHOD

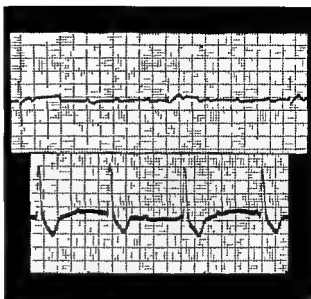
Healthy mongrel dogs of both sexes weighing between 9 and 15 kg, were anesthetized with pentobarbital. Respiration was maintained by an intermittent positive pump respirator through an endotracheal tube. The right chest was entered through the fourth interspace and the pericardium widely opened anterior to the phrenic. The atrioventricular node is located approximately 1 cm cephalad to the coronary sinus orifice along the base of the posterior leaflet of the tricuspid valve at the junction of the atrial and ventricular septa. The ventricular septum at this point was probed by a small gauge needle inserted through the right atrial wall. When the A V node or the conduction bundle is stimulated arrhythmias are produced and at this point approximately $\frac{1}{2}$ cc of 1 per cent procaine was injected. If a satisfactory block was not produced other injections were made but usually the procaine was limited to a total of 5 cc.

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Fig 1 Top EKG Dog 33 showing complete atrioventricular dissociation with A V nodal rhythm Bottom EKG Dog 29 showing idioventricular rhythm



current which in innumerable previous experiments has invariably fibrillated the normal dog's heart

RESULTS

Of the 8 dogs who had the S A node blocked with procaine as demonstrated by loss or depression of the P wave and marked slowing of the heart 4 developed ventricular fibrillation with faradic stimulation

On application of the faradic stimulus the 8 dogs which had procaine injected into the region of the node without producing the A V dissociation instantly developed persistent ventricular fibrillation as did 4 dogs which had no injections

A successful block of the atrioventricular node was produced in 22 dogs giving complete dissociation of the auricles and ventricles with an auricular rate of 140 to 180 and a ventricular rate of 24 to 60 Three of the dogs developed an A V nodal rhythm while in 19 an idioventricular rhythm developed which arose distal to the A V node presumably in the Purkinje fibers

In the 3 dogs with an A V nodal rhythm fibrillation occurred in one It seemed that some protection was offered here in that multiple stimuli were required to produce fibrillation

In the 19 dogs with an idioventricular rhythm ventricular fibrillation was not produced in a single instance by the faradic stimulation Likewise simultaneous stimulation on each side of the heart with 2 pairs of electrodes did not produce fibrillation in these circumstances

Table 1

BLOCK OF	RHYTHM	DOGS	FIBRILLATION	PFR CENT
—	S A	12	12	100
S A node	Auricular	8	4	50
A V node	A V nodal	3	1	66
A V node	Ventricular	19	0	0

DISCUSSION

This finding that anesthetization of the atrioventricular node increases the resistance of the ventricle to fibrillation suggests a role of the atrioventricular node in the production and maintenance of fibrillation. It is interesting that complete exclusion of the A V node is required for this full effect as procainization of the conduction system at the S-A node with the production of an auricular rhythm appears to offer some protection but less than that where an idioventricular rhythm is produced.

Weidmann⁷ has demonstrated that perfusion of cocaine and other anti-fibrillatory drugs stabilize the Purkinje fibers and thereby protect the isolated fiber from fibrillation. It is not believed that this is the mechanism of protection here where the anesthetic has been given locally rather than by perfusion. Those animals with procaine injected into the auricular and ventricular septa but not directly into the conducting system did not enjoy the protection received by those with a similar or less amount of procaine injected only millimeters away.

It has been suggested that the protection offered by the block is by preventing vagal effect. However if anything vagal stimulation should offer some protection as Hoff and Nahum³ demonstrated that acetyl β methyl choline chloride offered marked protection to the ventricles against fibrillation following electric shock.

Hoff and Nahum³ demonstrated that removal of the stellate ganglia and the adrenal glands enormously decreases the susceptibility of the heart to ventricular fibrillation following electric shock. Supporting work for this has been presented by Harris and Biseni⁴ who found sympathetic ganglionic blockade drugs to reduce the ectopic activity following ligation of the anterior descending coronary artery. There is of course the possibility that blockade of the A V node removed some of the sympathetic stimuli. However as the sympathetic nerves do reach the ventricle directly as well as through the conduction system it does not appear that their influence has been completely removed by the block performed above.

Shumacker and co-workers found that anesthetization of the S-A node gave marked protection from ventricular fibrillation in hypothermic dogs subjected to right ventriculotomy and manipulation of the interventricular septum either at relatively low or moderate degrees of hypothermia. They found a similar protection from dorsal sympathectomy or from the sympathetic blockade drugs but did not explain this phenomenon. It is interesting that S-A blockade does not give the degree of protection from electrical stimulation offered by an A-V blockade.

The exact mechanism of this particular process by which anesthetization of the A-V node affords protection from ventricular fibrillation has no definite explanation at present. Further studies of both the electrical phenomena and varying electrolyte concentrations may be of help.

SUMMARY

- 1 Procaine anesthetization of the atrioventricular node in dogs producing complete heart block gives protection from ventricular fibrillation from faradic stimulation.

- 2 Blockade of the S-A node offers a lesser degree of protection.

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CORONARY FLOW AND RESISTANCE IN THE DOG DURING TOTAL BODY PERFUSION*

RAYMOND C READ JOHN A JOHNSON AND C WALTON LILLHEI

The development of extracorporeal pump oxygenator systems^{1 2} which allow temporary bypass of the heart and lungs during intracardiac surgery has also provided valuable experimental methods for the investigation of the circulation in different organs under conditions of controlled cardiac output. The purpose of this report is to present studies of the coronary circulation in the dog during body perfusion at various flow rates.

METHOD

Ten small mongrel dogs averaging 9 kg in weight were selected for use in these experiments so that a wide range of body flow rates could be studied conveniently. These animals were anesthetized with intravenous sodium pentobarbital.

For these acute experiments a modified pump oxygenator³ was utilized consisting of arterial and venous finger pumps⁴ activating $\frac{1}{2}$ " rubber tubing connected with plastic adapters and $\frac{1}{4}$ " Mayon⁵ polyvinyl tubing to the bubble oxygenator. Oxygenation was accomplished by bubbling 100 per cent oxygen into the mixing tube of the oxygenator. Antifoam spray⁶ was added in small amounts to the debubbling chamber. The debubbling reservoir rested in a warming bath which maintained the temperature of the blood.

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¹Sigma Motor Company, Middleport, New York.

²Mayon Plastics Company, Hopkins, Minnesota.

³Antifoam A, Dow Corning Company, Midland, Michigan.

and of the animal constant throughout the experiment. The apparatus was primed with 500 to 700 cc of fresh heparinized donor blood. The venous pump was connected to the animal via plastic tubing and a #11 French polyethylene catheter inserted into both superior and inferior vena cava through the right jugular vein. The holes in this catheter were so placed as to lie peripheral to the right atrium. Arterialized blood was returned to the animal through a similarly sized catheter with a terminal orifice introduced proximally for about 3 cm into the right common carotid artery. Ventilation was carried out with a positive pressure respirator connected to a tracheal cannula. The chest was opened through a longitudinal sternal splitting incision. Both vena cavae were looped near the right atrium with umbilical tapes and the azygos vein was tied. The pericardium was opened and the main pulmonary artery was dissected from theorta and looped with a tape. Arterial perfusion was started and the tapes around the vena cavae were secured on the venous catheter thus diverting the venous return into the oxygenator. The venous pump had been started and adjusted to balance the outflow from the oxygenator reservoir. Soon after cardiac bypass had been started a right angled glass cannula was passed through an incision in the main pulmonary artery into the outflow tract of the right ventricle and secured there. The outflow of this system was allowed to drain through plastic tubing led into a vertical reservoir. Coronary sinus and Thebesian venous drainage into the right heart thus collected accounts for approximately 95 per cent of the total coronary flow.³

The coronary flow was measured by the slope of the pressure change at the bottom of the coronary outflow reservoir. A constant slope is indicative of a constant flow rate and the steeper the slope the higher the flow rate. All values obtained were the mean of 4 to 5 determinations. Mean aortic and vena caval pressures were measured through polyethylene catheters inserted into the appropriate femoral vessels. Right ventricular pressure was measured with a needle tip catheter inserted through the myocardium. All recordings were made on a Sanborn polyviso. The coronary reservoir was circulated into the oxygenator when necessary with another SigmaMotor pump. In some animals the heart was fibrillated and defibrillated electrically.

The total body perfusion rate was determined at the beginning and end of each experiment by direct calibration of the arterial pump utilizing a graduated cylinder and stop watch. The output of this arterial pump was not influenced by the resistance offered by the inflow catheters nor by varying vascular resistance in the animal. During the actual experiments arterial inflow rates also were measured with a Ludwig stromuhr. Excellent agreement between the two methods was obtained.

RESULTS

The total body perfusion was maintained for approximately 3 hrs in each animal. Thirty two different flow rates were studied ranging from 7 to 81 cc/kg body wt/min. The inferior vena caval pressure during perfusion varied between 3 and 8 mm Hg and in each case was not significantly different from the control readings. The pressure in the right ventricle was less than atmospheric when there was a siphonage effect created in the drainage tubing. At low (less than 20 cc/min) coronary flows however the pressure was the hydrostatic head to the level of the sternum from the heart. This

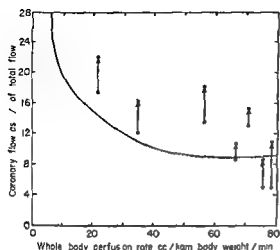


Fig. 1 The average relationship between percentage coronary flow and perfusion rate as well as the effect of ventricular fibrillation at 7 particular flow rates

right ventricular pressure ranged around 5 mm Hg and has been disregarded in the values for pressure difference across the coronary bed

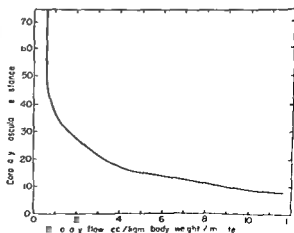
The pressure flow relationship of the coronary bed was essentially linear with an intercept on the pressure axis of about 18 mm Hg. There was a tendency for the coronary vascular resistance to decrease during perfusion. The proportion of the total body flow passing through the myocardium varied with the perfusion rate (Fig. 1). Thus at high perfusion rates (80 cc/kg/min) the mean coronary flow made up 8 per cent of the total and at low rates (10 cc/kg/min) 24 per cent of the total flow passed through the myocardium. However this relative increase was insufficient to prevent an absolute decline in flow at the lower perfusion rates.

Induced ventricular fibrillation in 7 instances increased coronary flow, the change being abolished on defibrillation (Fig. 1). When coronary vascular resistance was plotted against blood flow a hyperbolic relationship was seen with markedly increased values at very low flows and higher flow rates being associated with declining resistance (Fig. 2). The resistance showed a similar asymptote to infinity at low arterial pressures but a minimum was reached at slightly higher pressures with much higher pressures being related to an increase in resistance.

At autopsy the mean heart weight was found to be 1 per cent of the average body weight.

Fig. 2 The average relationship between coronary flow and coronary vascular resistance ——— units =
Blood pressure mm Hg

Coronary Flow cc/kg body weight/min



DISCUSSION

These studies show that under conditions of total body perfusion the bypassed heart receives a remarkably liberal coronary flow. When total body flow is reduced or if perfusion is prolonged the proportion of blood passing through the myocardium increases favoring the heart over other body organs. The flow through the essentially non-working heart is comparable to that accepted ($65 \text{ cc}/100 \text{ gm heart weight/min}$)⁶ for the normally functioning organ. This finding as well as the increase in flow associated with the presence of ventricular fibrillation are possibly explained by decreased intramural tension due to the absence of either normal function or contraction. This extravagant perfusion of the bypassed heart accounts for its excellent reaction to low flow body perfusions without increased susceptibility to ventricular fibrillation or other detectable damage. This tolerance has been observed repeatedly in the experimental and clinical experience with the bypass or low flow principle.⁶ This favoring of the heart during extracorporeal maintenance of the circulation is in direct opposition to the effect of total circulatory standstill where the heart unless resuscitated becomes the limiting factor in survival.⁶ The finding of a finite pressure gradient across the coronary bed when the flow has fallen to zero supports the hypothesis of Burton.⁷ The minimal resistances at very low body flows suggest that hypoxia may begin to affect the heart at that time and this factor with accompanying accumulation of metabolites doubtlessly contributes to the decreasing resistance after prolonged perfusion.⁸

SUMMARY

1 The coronary circulation has been studied in 10 dogs whose hearts were bypassed with artificial maintenance of the circulation for in most cases 3 hours using a pump oxygenator with perfusion rates of between 7 and 81 cc/kg/min .

2 Coronary venous return was drained from the main pulmonary artery and monitored continuously using an electromanometer.

3 The relationship between arterial pressure and coronary flow was approximately linear with an intercept of 18 mm Hg on the pressure axis.

4 The portion of the total body flow passing through the coronary bed varied between 1 and 27 per cent the higher proportions being associated with lower body perfusion rates.

5 Coronary resistance tended to decrease with duration of perfusion and in 7 instances was also uniformly lowered by the induction of ventricular fibrillation.

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RAPID DETECTION OF CARDIAC ARREST WITH ELECTRICAL RESUSCITATION*

K. WILLIAM LOMARK AND HENRY N. HARKINS

Cardiac arrest is now the chief cause of operating room mortality.¹ As surgeons we are justifiably concerned for operative mortality to continue to decrease. Methods for rapid detection and treatment of cardiac arrest must be developed.

It is impossible to predict in which patient cardiac arrest will develop.² It is most commonly seen in a healthy patient who is undergoing a major abdominal procedure. Cardiac arrest is not a major cause of mortality in the poor risk patient with cardiac irregularities in whom it would be suspected. Most commonly it is seen in a 61 year old male undergoing major abdominal surgery who is in good health and would be expected to survive the operation uneventfully.

As such it would seem logical to direct attention toward developing a method whereby cardiac arrest could be instantly detected in the routinely anesthetized patient. When detected, resuscitation could be started at once without the unknown time delay between its onset and recognition that exists today. In previous work³ a simple cardiographometer was developed for monitoring the QRS complex by using 2 electrodes taped to the chest. A built-in minimum rate warning alarm automatically sounded if the heart rate dropped below a pre-determined level. Using this apparatus to detect the cardiac arrest this experiment was undertaken to determine if the arrested heart could be resuscitated without cardiac massage using in its place an electrical stimulation applied across the axis of the heart through the unopened chest. The stimulating pulse used for creating artificial ventricular contraction is 5 to 10 milliseconds in duration, 100 volts in intensity and applied at a rate of 100 to 120 pulses per minute. The same electrodes that operate the cardiographometer are used for applying the stimulation across the chest.

*From the University of Washington School of Medicine Department of Surgery. This study was aided by a grant from the Washington Heart Association.

To simulate the type of cardiac arrest that is seen in the operating room mongrel dogs were given a uniform dose of intravenous pentobarbital (261 mg/kg of weight). In addition to this a varying amount of ether was administered through an intratracheal tube. The intratracheal tube was clamped and after cardiac arrest had occurred, trans thoracic electrical stimulation was applied to the chest. If ventricular contraction is manifested by a femoral pulse tracing did not occur coincidentally with the applied stimulation within 1 min, a conventional cardiac massage was done.

Figure 1 illustrates the type of 4 channel continuous tracing made on each animal. The tracing speed was one tenth of the normal rate for a cardiogram or 25 cm/sec. From analyses of these tracings the following information was obtained. Cardiac arrest was produced 45 times, 28 animals (65 per cent) were resuscitated by use of the trans thoracic stimulation and the remaining 17 animals (35 per cent) required cardiac massage. In this group death occurred in 2 animals. The first died on the second postoperative day from toxic brain changes. The second developed a spontaneous ventricular fibrillation following cardiac massage. Transient defibrillation was accomplished but could not be maintained. The animal died 1 hr after the onset of cardiac arrest. The over all survival rate in this series was 95 per cent.

The stimulation as applied across the chest did not produce ventricular fibrillation. In 1 animal the needle electrode was deliberately introduced through the chest wall into the myocardium. Ventricular fibrillation was

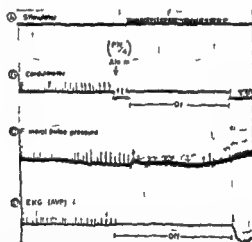


Fig 1 Typical 4 channel tracing showing A Application of cardiac stimulator pulse across the chest at 130 beats per minute. B Cardiometer* indication Drop of base line indicates point at which the minimum rate warning device sounds (in this case at 40 beats per minute) and the respirator is started. When stimulator is turned on the Cardiometer automatically stops functioning. C Femoral pulse tracing. At the irreversible rate point (1 N/4) the spontaneous heart rate is 40 with a low pulse pressure. Under the influence of the stimulator the heart rate instantly becomes 130/min and the pulse pressure gradually rises over a 1 min interval. At the end of this interval when the stimulator is turned off the spontaneous heart rate has become 120. D W E K tracing at 25 cm/sec. The instrument used has a capacity input requiring it to be turned off while the stimulation is applied.

*Trademark Physio Control Co

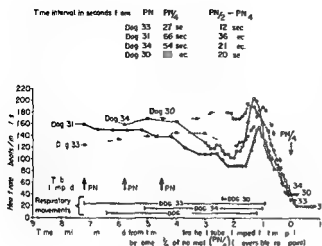


FIG. 2 Spontaneous respiratory movements are not seen in all animals as soon as the respirator is stopped and intratracheal tube clamped. Those deeply anesthetized and well oxygenated do not develop spontaneous respiratory movements for several minutes. Spontaneous respiratory movements stop as the heart rate reaches its maximum. This is followed by a precipitous decrease in heart rate. Time intervals $PN - PN_{1/4}$ and $PN_{1/2} - PN_{1/4}$ are measured after the pulse rate maximum has been reached.

instantly precipitated as seen on the monitoring oscilloscope. Thoracotomy with defibrillation and uneventful recovery followed.

In studying the pulse rate changes that occurred prior to cardiac arrest a fairly typical pattern developed. Figure 2 shows a composite graph of the pulse rate of 4 animals. It is to be emphasized that the pulse rates indicated on this graph were determined by differentiating succeeding 6 to 12 sec intervals.

The time differential was chosen carefully to give the greatest meaning to the graph. A time differential in seconds (Δx) that is too great yields a rate differential in heart beats a minute (Δy) that is too small to have significance for example

$$\lim \frac{\Delta y}{\Delta x} = 2$$

$$\Delta x \longrightarrow 30$$

A pulse rate change of 2 beats a minute has little clinical significance. Conversely if the time differentiation becomes too small the smallest rate differential detectable becomes so large as to become confusing rather than helpful. When

$$\lim \frac{\Delta y}{\Delta x} = 30$$

$$\Delta x \longrightarrow 2$$

we would know if the heart rate was 0, 30, 60, 90, 120, etc. But it is desirable to know the intermediate rates and therefore the following differentials were chosen

$$\lim \frac{\Delta y}{\Delta x} = 10$$

$$\Delta x \longrightarrow 6$$

$$\lim \frac{\Delta y}{\Delta x} = 5$$

$$\Delta x \longrightarrow 12$$

This yields detectable rate changes that have clinical meaning, namely 5 to 10 beats per minute.

The most significant finding was the extremely rapid fall that occurred in the pulse rate just prior to cardiac arrest. This pulse rate change varied between 140 and 240 beats per minute, and the principal change occurred during the last 30 sec. By preliminary experimentation it was shown that when the heart rate dropped below one-fourth of the normal rate, spontaneous resuscitation did not occur without a brief period of artificially induced ventricular contraction produced either by intrathoracic electrical stimuli

tion or cardiac massage. Since in all cases spontaneous respiratory movements stopped prior to this point being reached it was concluded that when the heart rate reached one fourth of normal (PN/1) a 100 per cent mortality would result in the animals without both artificial respiration and artificial ventricular contractions being produced. This point is arbitrarily called the irreversible rate point PN/1.

The slope of the line of this decreasing heart rate when plotted on a log-log scale gives a linear relationship with time during the 30 sec preceding the irreversible rate point. At the irreversible rate point a slope change occurs which is a break with the rate-time offering a mathematical correlation with the clinical observation that this point initiates cardiac arrest.

SUMMARY

Cardiac arrest was produced 15 times in mongrel dogs. Artificially induced ventricular contraction was produced by electrical stimulation in 28 of these and the remainder required cardiac massage. The pulse rate changes in the last 30 sec prior to cardiac arrest occur at a very high rate, and to be comprehended a cardi tachometer must be used. Respiratory failure occurs as the pulse rate reaches a maximum preceding the precipitous pulse rate decrease. When the pulse rate decreases to one fourth of normal spontaneous recovery does not occur without a brief period of artificially induced ventricular contraction. This artificially induced ventricular contraction can be produced in 65 per cent of cases by electrical stimulation without cardiac massage. If electrical stimulation is not effective within one minute artificial ventricular contraction must be produced by cardiac massage.

CONCLUSIONS

The pulse rate change curve immediately preceding cardiac arrest when produced by excessive anesthesia and anoxia is a characteristic one. When the spontaneous heart rate has reached one fourth of normal in the absence of spontaneous respiratory movements cardiac arrest is present. To be accurately detected at this earliest point a cardi tachometer with a minimum rate warning device should be used. When cardiac arrest is detected at this point 65 per cent of the animals can be resuscitated without the necessity of cardiac massage by using a proper type of electrical stimulation.

The mortality from cardiac arrest in experimental animals is much lower than that which exists in the operating room. This difference in mortality is probably due to the unknown time delay that exists between onset of cardiac arrest and its actual detection. The routine use in the operating room of a cardi tachometer with a minimum rate warning device would eliminate this delay.

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DIFFERENTIAL CARDIAC WARMING DURING HYPOTHERMIA*

JAMES R. JUDE, JEAN P. FAUTRUX AND LOUIS M. HAROUTUNIAN

The heart is less tolerant of anoxia than the rest of the body under moderate hypothermia. Lack of coronary blood flow is therefore a limit of the length of safe circulatory occlusion. The cold heart is also extremely sensitive to manipulation with ventricular fibrillation not infrequently the result. It can be postulated that if during general body hypothermia the heart were near normal temperature and the coronary blood flow maintained these problems would be obviated.

This investigation is of a technique of coronary perfusion and differential cardiac warming utilized to achieve this state.

METHOD

Fifty mongrel male and female dogs weighing 8 to 15 kg were used. Anesthesia was by endotracheal ether intermittent positive pressure with alveolar CO_2 maintained near 4 vol per cent and blood pH thereby normal.

The animal was wrapped in a special blanket† through which ice water was circulated. As cooling proceeded a thoracotomy was made sterily through the right fourth interspace. The aorta was isolated and a purse string of 3-0 arterial silk placed in its ascending portion $1\frac{1}{2}$ cm distal to the aortic ring and proximal to any branches except for the coronaries.

The coronary perfusion apparatus consisted of 500 cc of heparinized arterial blood (20 mg heparin) which had been freshly drawn from a healthy donor animal and kept at a constant temperature of 38°C. The perfusate bottle was attached at a height sufficient to deliver a pressure of 7½ mm Hg at the level of the ascending aorta. In some experiments the regular transfusion tube was attached to a coil of polyethylene tubing (volume 10 cc) contained in a basin of water at 38°C. From this differential warming basin the tubing ran a short distance directly to a #15 needle (short bevel with a 1 cm shaft) which was inserted through the purse string into the ascending aorta (Fig 1).

At 26–28°C the cavae were occluded and the aorta cross clamped distal to the perfusion needle. The coronary perfusion was begun and a 1 cm right ventriculotomy or auriculotomy immediately performed. Myocardial temperatures were taken with a #27 needle containing a thermistor at its tip. The interventricular wall was either massaged with the fingertip or stimulated with a suture. Close notice was made and recorded of the manner in which the heart recovered function after 10 to 20 min occlusion. If no indication of return of cardiac function was evident in 3 min massage and resuscitation were initiated.

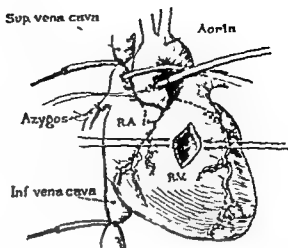
RESULTS

A right atriotomy was performed with differential cardiac warming by coronary perfusion on 8 dogs. No ventricular fibrillation occurred and all

*Department of Surgery, The Johns Hopkins Hospital and University, Baltimore, Maryland. This study supported in part by funds provided under Contract AF 41(657)30 with the USAF School of Aviation Medicine, Randolph Field, Texas.

†Fabricated and provided by David Clark Mfg. Co., Worcester, Mass.

Fig 1 Method of coronary perfusion and differential cardiac warming during hypothermia



animals survived rewarm. Five dogs were long term survivors. The return of cardiac function on the re establishment of the circulation was good in every instance. A good return of cardiac function was considered to be a blood pressure of at least 50 mm Hg with a good pulse pressure within 1 min after the opening of the aorta. A poor return was one which would require 3 min or more to reach this state.

Differential cardiac warming by coronary perfusion with a right ventriculotomy was performed on 11 dogs. Ventricular fibrillation occurred in only 1 animal and that with the performance of the ventriculotomy. With the coronaries being perfused the animal was readily reverted to a normal rhythm with a single electroshock after a 3 min period of fibrillation and without the use of massage or drugs. The 1 unsuccessful dog in this group was due to right heart failure on the return of circulation after 17 min caval occlusion. Ten animals were long term survivors.

Thirty one control animals had a standard 10 to 11 min caval occlusion with a 4 cm right ventriculotomy. The aorta was not occluded. Eleven animals suffered ventricular fibrillation either with the ventriculotomy or during the occlusion period. The heart did not resume its function and suffered right heart failure in 6 dogs. Seven dogs had a poor and slowly returning cardiac function on the re establishment of the circulation. All except 5 of those 24 animals succumbing to right heart failure or ventricular fibrillation were resuscitated by the use of massage, drugs and electroshock as needed. Only 7 dogs had a good return of heart beat and blood pressure without assistance.

With a coronary perfusion pressure of 75 mm Hg gravity there was utilized 25 to 30 cc of perfusate blood per minute. The gravity flow was completely selective being greater or less as dictated by the myocardial need.

On perfusion with normothermic blood without the heat exchanger coil the myocardial temperature was elevated to 30 to 31°C. With the use of the heat exchanger the myocardial temperature was elevated to 34 to 36°C. There was little difference in the temperatures of the various portions of the heart.

In general the entire procedure (cooling, occlusion and rewarm) required about 2 to 2½ hrs and the animals were awake and standing in their cages within a few hours of completion. The animals received no antibiotics and no parenteral blood or fluids.

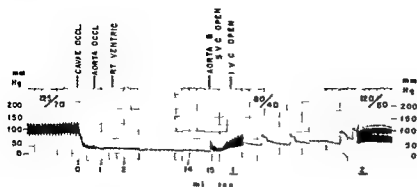


Fig 2 Effect of coronary perfusion with warm blood on return of cardiac function at 28 C. Fifteen minute occlusion

Selective Coronary Perfusion Dog 158 28 C

There was no indication of central nervous system damage in any of the animals rewarmed after circulatory occlusions of up to 20 min

DISCUSSION

Coronary perfusion with differential cardiac warming reduced the incidence of ventricular fibrillation during the cardiac occlusion practically to zero in this series. Cookson and Costas Durieux¹ and Shumway *et al*² have also emphasized the use of coronary perfusion during occlusion to prolong the possible period of cardiac occlusion.

The culpability for ventricular fibrillation occurring on reestablishment of the circulation can not be placed on the hypothermia *per se* as the heart both cold and more or less hypoxic depending on the length of circulatory occlusion may not accept the work load suddenly thrust upon it and right sided heart failure results. Attempted resuscitation on the irritable myocardium then almost invariably results in ventricular fibrillation. This fibrillation is secondary to heart failure and not directly due to cold. The effect on the return of cardiac function of coronary perfusion with warm oxygenated blood is shown in the blood pressure and pulse tracing of Figure 2 as compared to that without coronary perfusion or cardiac warming in Figure 3.

Selective coronary perfusion of normothermic blood by gravity pressure has been utilized here because it offers arterial blood to the coronaries when the myocardium requires it at a near normal pressure and temperature. Our

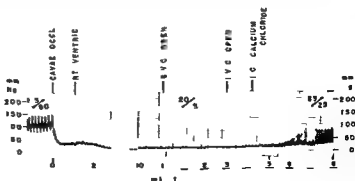


Fig 3 The return of cardiac function in the control dog at 28 C. Fifteen minute occlusion

initial experiments in which we used a finger pump to supply the coronary perfusion gave an alarmingly high incidence of fibrillation. The cause was found to be due to widespread myocardial hemorrhage. Arteriovenous oxygen studies revealed that high coronary flows were not necessary in the cold empty beating heart. With selective coronary perfusion 25 to 30 cc/min was sufficient flow.

Clinically the use of a perfusate needle in the ascending aorta would allow the decision for use of coronary perfusion in any particular case to be delayed and yet rapidly be applied if desired without addition of dissection. Adequate flow is obtainable through a modified #15 needle.

Since hypothermia *per se* is of added risk to the operative procedure it is advisable to rewarm as quickly as possible. Presently conventional methods of cooling and rewarming require good blood flow for heat conduction inward or outward. A prompt and effective return of blood pressure following occlusion has the beneficial effect of enabling rapid elevation of the temperature.

This method has been successfully used clinically by Dr. Henry T. Bahnon in closing an aortic septal defect with anomalous venous return in a 7½ year old male. Myocardial irritability was absent and there was immediate return of excellent cardiac function.

SUMMARY

Selective coronary perfusion of warm arterial blood reduced practically to zero the incidence of both ventricular fibrillation during cardiac occlusion and cardiac failure on re-establishment of normal circulation.

In 19 dogs with coronary perfusion and a right ventriculotomy or ventriculotomy of 10 to 20 min duration at 26 to 28°C there occurred only one instance of ventricular fibrillation which was readily reverted to a normal rhythm. Only 1 dog did not have an immediate return of good cardiac function.

In 31 control dogs with a right ventriculotomy for 10 to 11 min at 25 to 28°C there were 11 instances of ventricular fibrillation. In addition 13 dogs had either no or poor return of cardiac function.

*We are grateful to Dr. Alfred Blalock for his encouragement and advice in this study.

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Coronary Arteries and Great Vessels and Angiography

STUDIES IN VENTRICULAR FIBRILLATION IN THE PIG EVALUATION OF AN ANTIFIBRILLATORY AGENT TESTED BY CLOSED CHEST CORONARY ARTERY OCCLUSION*

JOSEPH J. GARAMELLA LYLE J. HAY AND JAMES G. ANDERSEN

In spite of the vast amount of experimental work that has been performed on coronary artery disease there still remains a great deal of confusion and controversy relative to the benefits of various revascularizing procedures and medications. Experimental work has been dubiously accepted or refuted sometimes simply ignored principally because of the wide discrepancies reported on the consequences of acute coronary artery ligation in the dog our heritage dating to 1698 when this procedure was first performed by Chirac¹

The mortality differences reported following coronary artery occlusion are readily understood if one will briefly survey the multiple variables present in a seemingly simple experiment. These include 1) experimental animal (usually the dog)—health age size 2) premedication 3) anesthetic agent—respirator—pH 4) operator 5) site of ligation 6) period of open chest, 7) period of follow up i.e. time of death 8) season 9) temperature of animal 10) diet 11) coronary artery pattern i.e. left or right preponderance, 12) intercoronary development magnitude.

With the object of minimizing the influences of premedication anesthesia and the open chest upon experimental acute coronary artery occlusion a reliable method of closed chest coronary artery occlusion has been developed. Furthermore because of the variability of the coronary artery anatomy in the dog the present studies were performed on the pig. The anatomical factors rendering the pig more suitable for this type of study have been given by Blumgart *et al*.²

It is therefore the purpose of this report to present first a method of acute closed chest coronary artery occlusion in the pig and second the application of this method in testing an antifibrillatory agent Ro 2 5803 † Ro 2 5803 is 2,6-Bis(1-piperidylmethyl)-4-(α , α -dimethylbenzyl) phenol dihydromide (Fig 1). According to Schallek and Wong³ Ro 2 5803 is more toxic than quinidine in rats and mice it is more potent than quinidine in the isolated (rabbit) auricle test and equi potent in preventing auricular fibrillation in dogs.

From the Jay C. Phillips Research Laboratory of Mount Sinai Hospital and the Department of Surgery, University of Minnesota, Minneapolis, Minnesota. Supported in part by the Minnesota Heart Association, American Heart Association, Cardiac Research Committee, Minneapolis, Minnesota, National Heart Institute (Grant 2610) of the National Institutes of Health, Public Health Service Fund for Research on Coronary Heart Disease, University of Minnesota.

*Made available for this study by Dr. M. J. Schiffrin, Hoffmann-La Roche, Inc., Nutley,

Ro 2-5803

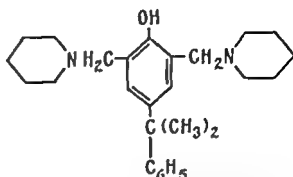


Fig. 1 Chemical structure of Ro 2-5803

2,6-Bis (1-piperidylmethyl)-4
(α,α -dimethylbenzyl)phenol dihydrochloride

METHOD

Young stockyard pigs weighing between 15.6 and 30 kg were anesthetized with intraperitoneal chloral hydrate 300 mg/kg of body weight and intravenous nembutal 3-4 mg/kg of body weight. Animals were intubated and ventilated with compressed air by an automatic respirator. Using aseptic technique left thoracotomy was done through the fourth or fifth interspace and the pericardial sac was opened. At the bifurcation of the left coronary artery the left anterior descending branch was dissected free. The coronary artery occluding apparatus was installed at the origin of the left anterior descending branch. This mechanism* consists of a plastic snare which is passed around the coronary artery with ample slack to avoid compression of the vessel. The relationship of the snare to the axis of the vessel is maintained by a plastic platform which overrides the vessel to be occluded and which is sutured to the adjacent epicardium. An adjustable armature attached to the plastic platform can be passed under the main pulmonary artery and sutured to the adventitia for stabilization. The plastic cord is enclosed within a flexible plastic cable which passes from the coronary artery through the thorax and is carried to the exterior between the ribs, muscles, subcutaneous tissue and finally through the skin where a control unit is fixed in position. The control unit can be implanted under skin, muscle or in the thorax. Manual self-winding or magnetically controlled movements of the control side of the occluding apparatus provide either acute one staged or chronic progressive vascular or tubular occlusion (Fig. 2).

In these experiments 1½ to 21 hrs after installation of the occluding apparatus and chest closure acute one staged occlusion of the anterior descending branch of the left coronary artery was performed. Electrocardiograms were taken before, during and after coronary artery occlusion.

RESULTS

In 15 successful control studies this method of closed chest coronary artery occlusion was performed. All 15 pigs developed irreversible ventricular fibril-

*Minneapolis Honeywell Regulator Company

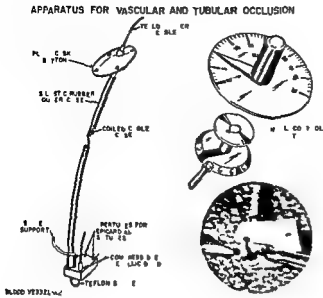


Fig 2 Schematic representation of apparatus for acute and chronic occlusion. Inset (right lower corner) shows the character of vascular occlusion from a removed specimen

lution confirmed by electrocardiography within $11\frac{1}{2}$ to $27\frac{1}{2}$ min. These results are in keeping with previous studies.²

In 18 pigs given Ro 25803 (0.57 to 8.8 mg/kg of body weight) intravenously 5 to 9 min before acute closed chest coronary artery occlusion 10 developed ventricular fibrillation within 2 to $4\frac{1}{2}$ min. Of the remaining 8 pigs 7 survived and were sacrificed 1, 1, 6, 7, 8, 12 and 53 days following coronary occlusion. The 8th pig in this group was last seen alive $21\frac{1}{2}$ hrs following acute coronary occlusion and was found dead in the kennel the following morning; extensive bilateral pneumonia was found at autopsy. The survival times of control and treated pigs are summarized in Figure 3. In autopsies of all animals in the control and treated groups complete occlusion of the left anterior descending branch was confirmed in each instance. All the hearts of animals dying of ventricular fibrillation, both control and treated pigs, were contracted and hard.

Fig 3 Survival times of the control group and the drug-treated group are charted.

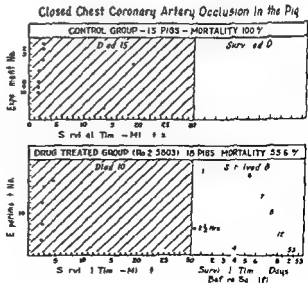
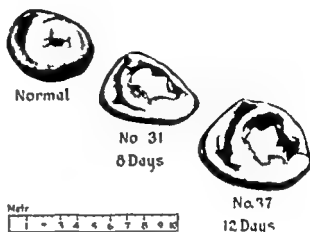


Fig 4 Transverse sections of pig hearts are shown. Note the uniform thickness of the ventricular wall and septum in the normal heart. Specimens number 31 and 37 were removed from drug-treated pigs which had survived acute closed chest coronary artery occlusion of the left anterior descending branch of the left coronary artery. The ventricular myocardium including the anterior portion of the septum supplied by this vessel demonstrates marked thinning and necrosis.

Pig Heart Infarcts Following Coronary Artery Occlusion



The hearts of the drug treated pigs surviving the acute coronary occlusion and subsequently sacrificed showed in each instance extensive transmural infarction in the area of distribution of the occluded vessel (Fig 1).

Some effects of the drug noted prior to coronary occlusion include 1) tranquilizing change constant 2) tachypnea 3) hypotension 4) pupillary dilation 5) electrocardiographic alterations (tachycardia pulsus alterans bigeminal rhythm sinoauricular block atrioventricular block T wave changes S wave changes). Optimum dosage toxicity and mechanism of action of the drug have not been established.

SUMMARY

- 1 A simple method of closed chest coronary artery occlusion is presented.
- 2 Fifteen control pigs subjected to closed chest acute coronary artery occlusion died of ventricular fibrillation in $11\frac{1}{2}$ to $27\frac{1}{2}$ min (100 per cent) whereas in 18 pigs treated with antifibrillatory agent Ro 25803 9 survived (50.5 per cent mortality). These differences are statistically significant at the one per cent level.
- 3 Further study relating to dosage toxicity and mechanism of action is indicated.

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THE EFFECTS OF GRADUAL OCCLUSION OF THE CORONARY ARTERIAL CIRCULATION IN DOGS AND PIGS*

ALBERT B. LOWENFELS, CHARLES G. NEUMANN, WILLIAM H. WADSWORTH, JR., JERROLD VON WITTEL, JEFF W. LORD, JR., AND J. WILLIAM HINTON

In connection with a previously described operation for revascularization of the heart with a pedicled skin flap a new method for producing gradual coronary arterial occlusion was evolved.¹ A small stainless steel spring clamp with jaws separated by surgical gut was placed around the coronary artery. After a period of time the gut was slowly absorbed allowing the clamp to occlude the coronary artery.

It was felt that this clamp might provide a useful method to study the effect of chronic obliteration of the coronary arterial circulation. Naturally occurring coronary collaterals might be expected to develop during the gradual occlusion which would afford considerable protection to the heart when closure finally became complete. Others have carried out somewhat similar investigations using different methods of occlusion.²⁻⁵

The authors thought that the use of delayed, self closing clamps would give insight into the amount of occlusion which a heart unprotected by any surgical method could withstand. It was felt that the mortality following gradual occlusion of a major coronary artery might be appreciably less than the figure of 70 per cent reported by Beck and Leightner⁶ for ligation of the anterior descending branch of the left coronary artery.⁴

METHOD

The clamp used was constructed of hard tempered stainless steel wire SMO 18-8 type 302 having a diameter of 0.025 in. This type of steel has been shown to be non reactive in the tissues. The wire was used to fashion an 8 coil spring which activated the clamp. The bends in the remainder of the clamp were placed with a set of hand pliers. In the fully closed position, the clamp will withstand a pressure of 300 mm. Hg. and prolonged exposure to the arterial pulsation was found not to weaken the spring mechanism.

This clamp was applied around a blood vessel with the jaws of the clamp held apart by a bolus of surgical gut. After contact with the body fluids the gut was slowly absorbed and the jaws of the clamp occluded the vessel. Fifty turns of 00 plain surgical gut were used in most instances although in a few clamps 00 chromic gut was used to separate the jaws.

It has been found that when the clamps were buried in the pericardial sac they closed in a period of approximately one month.

In the canine experiments healthy adult mongrel dogs weighing from 10 to 20 kg. were used. The animals were anesthetized with nembutal using 30 mg./kg. intubated and placed in the lateral position with the left side up. Permost Ventilation was maintained with a mechanical insufflator and room air.

An incision in the fourth intercostal space was used to expose the heart. The pericardium was opened and a Blalock clamp placed on the auricular

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Fig. 1 Photograph of self closing delayed action arterial clamp. The upper picture shows how a bolus of surgical gut keeps the jaws of the clamp apart. The lower picture reveals how the jaws close around a simulated blood vessel after absorption of the gut. The scale is in centimeter.



appendage to give better exposure of the base of the left coronary artery. This was dissected out and a clamp was placed either above the bifurcation or else clamps were placed on the major branches just below the bifurcation. An attempt was made to visualize the septal artery and it was ligated when ever possible. The chest was closed, the pleural space aspirated and the animal given an injection of penicillin in a dose of 300 000 units.

In animals later subjected to right coronary arterial occlusion a similar technique was used after a time interval of at least one month. An effort was made to place the clamp on the base of the right coronary artery before branches are given off.

A group of 6 pigs was also prepared in a similar fashion. Most of these animals required tracheotomy for intubation and these animals were maintained on inhalation anesthesia using cyclopropane or ether.

Postoperatively, the animals were observed and repeated electrocardiograms were obtained. Progress of closure of the clamps was studied with serial x rays. Some of the dogs were subjected to coronary arteriography using a catheter introduced through one of the carotid vessels.

The surviving animals were sacrificed from 1 to 18 mo. after operation. At the time of sacrifice careful observation was made of the appearance of the heart, presence of infarcts and status of the clamps. The heart was injected with a 20 per cent suspension of bismuth oxychloride and sections taken for microscopic examination.

RESULTS

Thirty two dogs were prepared as described above. At the time of death or sacrifice 24 animals were found to have total occlusion of one or more major coronary arteries. The results for this group are listed in Tables 1 and 2. The group of 8 animals in which the clamps did not close sustained a considerable degree of partial occlusion. This was estimated as high as 90 per cent for some of the arteries.

Table 1 Mortality Following Gradual Coronary Arterial Occlusion

LOCATION OF OCCLUSION	NUMBER OF DOGS	DEATHS	MORTALITY
Left main coronary artery	12	11	92%
Left circumflex and anterior descending branches at bifurcation	3	0	0
Left circumflex branch	3	1	33%
Left anterior descending branch	2	1	50%
Right coronary artery*	6	1	17%

*Five dogs in this group had previous operations on the left coronary artery (See Text)

Table 2 Results in Dogs Surviving Gradual Coronary Arterial Occlusion

OCCLUSION SUSTAINED	NUMBER OF DOGS	INFARCTION
Left and right main coronary arteries	1	0
Left circumflex and anterior descending branches at bifurcation	3	0
Left circumflex branch and right coronary artery	1	0
Left circumflex branch	1	0
Left anterior descending branch	1	1
Right coronary artery	3	1

Of 12 dogs which were prepared with a single clamp applied to the base of the left coronary artery 11 died within a month of operation. One animal survived and a month later a second clamp was placed on the base of the right coronary artery. Several postoperative electrocardiograms obtained on this animal were interpreted as normal. He died 14 months after the second operation following general anesthesia. Autopsy showed that the entire left coronary artery including the septal branch was occluded as was the right coronary artery. Injection with bismuth oxychloride suspension via the anterior descending branch of the left coronary artery outlined an essentially normal coronary arterial tree. No infarcts were present either grossly or microscopically.

Three animals were prepared with separate clamps placed around the anterior descending and the circumflex branches of the left coronary artery. These animals all survived total occlusion of the left coronary artery. Of 3 dogs with a single occluding clamp on the left circumflex branch 2 survived. One of 2 dogs with occlusion of the anterior descending branch of the left coronary artery survived.

Five out of 6 dogs survived occlusion of the right coronary artery. This group included 1 dog with additional occlusion of the left main coronary artery, another with occlusion of the circumflex branch of the left coronary

artery and 3 dogs in which the left coronary arterial clamps had failed to close. Of the surviving animals 1 had a 3 cm infarction of the anterior wall of the right ventricle. Arteriograms taken on 2 of the animals in this group showed an extensive collateral circulation developing between the occluded right coronary artery and the patent left coronary artery.

Table 2 shows the results in dogs surviving gradual coronary occlusion. Of the 10 surviving animals only 2 showed gross or microscopic evidence of infarction.

In addition to the group of dogs described above 6 pigs were studied. Three animals died suddenly 28, 15, and 66 days after operation. Two animals were found to have complete occlusion of the left main coronary artery without infarction and a third pig had occlusion of the anterior descending branch also without infarction. It is presumed that these animals developed ventricular fibrillation at the time of death.

In 2 large pigs partial erosion of the clamp through the vessel wall with subsequent recanalization of the coronary artery was noted.

One animal survived occlusion of the right coronary artery. At the time of sacrifice 9 months after operation a 2 by 3 cm infarct was found on the anterior wall of the right ventricle near the base.

DISCUSSION

A distinct difference can be noted between the effects of acute and chronic occlusion of the coronary arteries. It appears that when the coronary arterial system of the dog is subjected to gradual obliteration by a suitable method a significant and useful collateral circulation develops. This is corroborated by the high survival rate, minimal number of infarcts, and the lack of electrocardiographic changes. This difference between acute and chronic occlusion of the coronary arteries might be due to one or more of the following factors: 1 Increase in blood flow from venous system to capillary bed, 2 Increase in blood flow in intercoronary collateral circulation, 3 Increase in extracardiac collateral circulation, 4 Decrease in incidence of ventricular fibrillation.

Of these intercoronary anastomoses would appear to be the most likely protective mechanism. Evidence for this is seen in the arteriograms which show large intercoronary anastomoses connecting the coronary artery beyond the point of occlusion to another artery. That there is a limit to the ability of the heart to respond to gradual occlusion is brought out by the fact that 11 out of 12 dogs with a single clamp placed on the base of the left coronary artery died.

Wiggers⁶ has pointed out that intercoronary anastomoses are frequently present between the 3 principal coronary arteries in the dog, but that their presence in normal human hearts is controversial. In view of this one can doubt the wisdom of using the results of experiments on the dog's heart as the valid basis of making prediction for the course and surgical treatment of human coronary arterial disease.

The lack of naturally occurring intercoronary anastomoses in the porcine heart as pointed out by Eckstein⁷ would seem to make this animal more suitable for studies dealing with myocardial revascularization. In addition the anatomic arrangement of the coronary arterial system of the human and pig heart is quite similar. However, continued growth even with the Hormel

Institute midget pigs which were used in 1 experiments makes long term study difficult

Erosion of the present style clamp through the large coronary arteries of the pig was disturbing and a slightly different style clamp is being investigated in the hope of obviating this problem

SUMMARY

Using a slowly closing stainless steel clamp whose jaws are initially separated by surgical gut a group of dogs and pigs were subjected to chronic coronary arterial occlusion. The canine heart can withstand a high degree of occlusion if this is carried out in a gradual fashion over a period of about one month.

Evidence seems to point to intercoronary anastomoses as the source of blood for the occluded areas.

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DETERMINATION OF MYOCARDIAL BLOOD FLOW UTILIZING IODINATED (I^{131}) HUMAN SERUM ALBUMIN*

WALTER S HENLY OSCAR CREECH JR CECIL M COUVES MARY C MORGAN DON W CHAI MAN AND HERBERT C ALLEN, JR

The lack of a satisfactory method of determining myocardial blood flow in the intact animal has limited investigation of medical treatment of coronary artery disease and of surgical procedures designed to augment the coronary circulation. This report is concerned with a new method of determining myocardial blood flow in the intact animal. The method is based upon the rate of return of radio iodinated (I^{131}) human serum albumin to the right

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heart after injection of a known quantity of RISA into the left heart. In the preliminary investigation RISA was injected through a thoracotomy incision directly into the left ventricle of the dog while taking a continuous sample of blood from the right ventricular outflow tract. Later experiments were performed during cardiac catheterization in the intact subject.

METHOD

Cournand cardiac catheters (#8) are placed via the external jugular vein into the pulmonary wedge and the right ventricular outflow tract under fluoroscopic control. The blood pressure and the ECG are recorded during the determination. Cardiac output is determined by the direct Fick principle immediately preceding and following the myocardial blood flow determination. Polyethylene tubing (PI 205) 30 ft. long filled with heparinized physiologic saline solution is connected to the catheter in the right ventricular outflow tract and withdrawal of blood from the heart at a constant rate for 60 sec. is begun. A known quantity (200 to 750 microcuries) of RISA is injected into the pulmonary wedge in 1 sec. using an automatic injector. An immediate radiodilution curve is obtained by monitoring the tubing with a scintillation counter and recording with an Esterline Angus Graphic Meter. Immediately following the procedure the polyethylene tubing is rescanned for a more accurate quantitative determination. The residual radioactivity in the injection system is determined and the corrected quantity of RISA injected into the pulmonary wedge is calculated.

RESULTS

During the first minute following injection the changing concentration of RISA in the right ventricle could be determined giving a composite radiodilution curve of a typical pattern (Fig. 1). By simultaneous sampling from the right ventricle, coronary sinus, superior vena cava and inferior vena cava the components of this dilution curve were apparent (Fig. 2). In all animals the superior vena caval return did not occur until more than 5 sec. following the appearance of RISA in the right ventricle. Thus the radioactivity during the first 5 sec. of the dilution curve is contributed by the myocardial circulation and its rate of appearance in the right heart is a measure of myocardial blood flow. Ligation of the left main coronary artery significantly reduced the appearance rate. Immediate ligation of the coronary sinus produced no significant alteration in the rate of flow. The reproducibility of the method was demonstrated in 20 dogs using the open chest technique. Similar radiodilution curves could be obtained by injection of RISA into the pulmonary wedge in the intact animal.

To quantitate the myocardial blood flow in cc per minute 2 separate dilutions must be considered. The injected RISA is mixed first in the left heart. The dilution from the pulmonary wedge to the left atrium is insignificant. In the dog the injected volume has been large in comparison to the stroke volume and the dilution of RISA occurs over a 5 sec. period. During this time the RISA is diluted by a volume of blood equal to the cardiac output during the dilution period ($CO/sec \times t\ sec$). In the adult human the volume of the injected dose is small in comparison to the stroke volume and mixing is assumed to occur with a single beat of the heart. Hence a volume of blood of definite concentration of RISA is ejected from the left ventricle

NORMAL DOG RADIODILUTION CURVE

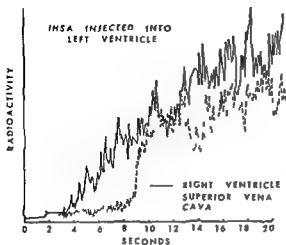


Fig 1

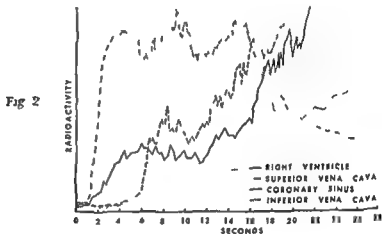
RADIODILUTION CURVE COMPONENTS
17 KG DOG

Fig 2

A second dilution is constantly occurring on the right side of the heart. This is the same regardless of the size of the animal and is equal to the volume of blood passing through the right ventricle during the sampling period. At any instant the quantity of RISA in the right heart will represent a definite volume of coronary flow. From the previous concepts the following formulae were derived in order to determine quantitatively myocardial blood flow. In the human

$$MBF = \frac{(CO/sec) (\text{stroke volume}) (\text{avg conc RISA})_{t \rightarrow \infty} (60)}{RISA_{pw}}$$

In the dog

$$MBF = \frac{(CO/sec)^2 (\text{avg conc RISA}_{tw})_t (300)}{(RISA)_{pw}}$$

Where $RISA_{pw}$ = microcuries of RISA injected into the pulmonary wedge

$RISA_{tw}$ = microcuries of RISA in the right ventricle

CO/sec = cardiac output/second

$t = 5$ seconds

MBF = Myocardial Blood Flow in cc/minute

Table 1 Myocardial Blood Flow—Animal Data

BP	MBF	% CO
130/110	101 cc	57%
115/100	285 cc	54%
130/115	183 cc	86%
50/40	215 cc	33%
140/100	100 cc	56%

Table 2 Myocardial Blood Flow—Human Data

	MBF	% CO
Normal ECC		
WM 33	316 cc/min	40%
CM 17	311	61%
CM 30	261	80%
Abnormal ECC		
CM 59	123 cc/min	28%
WM 61	171	51%
WM 62	163	44%

Table 3 Myocardial Blood Flow Calculated vs Measured Flow

BP	MBF (CALCULATED)	BP	MBF (MEASURED)
130/115	131 cc/min	105/95	993 cc/min
140/100	100	60/50	73
130/110	707	70/55	45

The results obtained from preliminary determinations in dogs and humans are in the predicted range of myocardial flow and support the original assumptions concerning the manner of dilution of the RISA (Tables 1 and 2). To compare the calculated myocardial blood flow with a direct measurement of coronary sinus and thebesian flow into the right ventricle extracorporeal circulation was instituted with a pump-oxygenator the pulmonary artery was occluded and venous return to the right ventricle was collected and measured. The results correlated well with those obtained using RISA (Table 3).

SUMMARY

In summary a new method of determining myocardial blood flow utilizing iodinated (131) human serum albumin has been described. It is applicable to the intact individual during routine cardiac catheterization with an adequate margin of safety. With additional refinements in technique the method should prove useful in evaluating problems associated with coronary artery disease.

A PRELIMINARY REPORT ON A RADIOISOTOPIC METHOD FOR MEASURING CEREBRAL BLOOD FLOW*

PAUL H. CRANDALL AND BENEDICT CASSIN

The need for a method of study for portions of the cerebral circulatory system is, we believe, fulfilled by the radioisotopic angiogram proposed here. At present the principal methods for estimating cerebral circulation are the appearance of the contrast angiogram and the nitrous oxide method. The nitrous oxide technique yields a quantitative over all value of blood flow quoted for the normal young adult as 51 cc (± 12)/100 gm. brain/min.¹ The contrast angiogram gives a serial radiographic record after the intra arterial injection of various contrast media but does not lend itself to quantitative measurement of blood flow. The method proposed here has been used as a supplement to the contrast angiogram of the carotid torcular circulation for the specific purpose of determining cerebral blood flow and circulation time.

METHOD

All patients chosen for this study were undergoing diagnostic carotid angiograms for various clinical reasons. The agent used was radioactive iodinated (I^{131}) serum albumin (RISA) the safety of which has been well attested. The amounts used were well below any hazardous level.

A marker was placed on the patient's scalp either on theinion or at the junction of the external occipital crests with the sagittal suture. Using local anesthesia a #19 or #20 gauge spinal needle was inserted percutaneously into a common carotid artery. A radiographic record was then made following intra arterial injection of 8 cc to 10 cc of Hypaque. These pictures were used for qualitative correlation with the subsequent radioactive angiogram and, also, to determine accurately the position of the torcular Herophili in relation to the external scalp marker.

Following the contrast study a radioactive blood flow study was made. The equipment consisted of a well collimated, shielded scintillation counter that recorded onto a scintiscaler and Sinborn Visocordette mounted as a portable unit with the counter on a movable arm so that the entire unit may be adapted to many locations and positions (Fig. 1). The counter aperture is 1 in. in diameter. With the patient's head in a lateral position the counter aperture was placed directly over the torcular as determined by measurements derived from the previous study and scalp marker. In addition using a simple goniometer one arm of which was on a line between the lateral limbus of the eye and the external auditory meatus the counter was then placed at an angle of 30° with a plane parallel to the Frankfort plane.

After a background activity of 10 sec. or more was determined a dose of 10 to 20 microcuries of RISA was injected in the common carotid. The variations in activity were recorded in counts on the Sinborn Visocordette for 16 to 18 sec. After an equilibrium was reached (5 min. plus) this value was recorded for 10 or more sec. and a simultaneous blood sample from a suitable vein was drawn to determine blood volume. The dilution curve was then plotted in terms of counts per 2 sec.

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Fig. 1 Recording unit consisting of a scintillation counter, scintiscaler and Sanborn Visocardiette

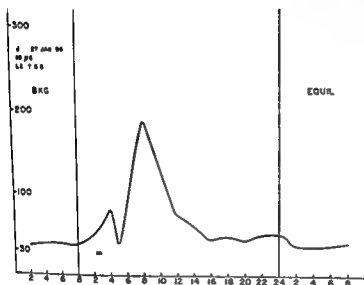
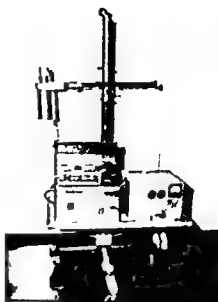


Fig. 2 Dilution curve over torcular Herophili in a normal young adult

Figure 2 represents the usual response in a normal young individual. It will be seen that the large curve does not return to the level of the background activity and hence the remainder of the dilution curve is assumed to be a simple exponential and is therefore extrapolated. The difference which is due to the effect of circulation other than that in the torcular is so small that minimal error is possible by this extrapolation. Using an equation

$$\text{Cerebral flow} = \frac{\text{Equilibrium}}{\text{Total count of curve}} \times \text{total blood volume} \times 60$$

the cerebral blood flow through the torcular is obtained.

The size of the dose of RISA is important only in obtaining a curve that is as high as possible without exceeding the counting ability of the Sanborn Visocardiette; the higher the curve, the more accurate the determination. We have found it useful to use 20 microcuries in 2 cc of RISA as the usual dose to be administered.

DISCUSSION

The scintillation counter in effect subtends a beam the angle of which is 26° . When placed in the specified anatomical position, it sees the following large blood vessels: the torcular Herophili, the vertebral artery and basilar venous plexus and the carotid arteries and cervical veins. Considering the RISA as a bolus of material passing through the system immediately after injection, the carotid arteries, the torcular and the cervical veins are seen in sequence but sufficiently spaced in time so that the 3 components can be measured.

Corroboration of this effect was noted in examinations of 2 patients who had thrombotic occlusion of an internal carotid artery. When the RISA was injected in the common carotid below the site of thrombosis, an initial rise during the first second occurred but was followed by a fall to the pre-existing background level. A second rise, 12 sec after injection, represented a cervical venous flow from the external carotid circulation. The main curve ordinarily contributed by the torcular was absent. In the majority of patients we have been able to distinguish these 3 portions of the curve.

The effect of distance must be evaluated. In considering a point source in front of the counter, the value for radiation falls off as the inverse of the square of the distance, so that in this situation the layer of RISA nearest the counter contributes by far the largest part of the radiation curve. Since in the injection phase the RISA is passing as a bolus through the system and since the torcular is such a large vascular structure closely applied to the skull and near to the counter, the main dilution curve can be attributed to torcular flow. The accuracy for determination of blood flow in the peripheral circulatory system depends upon the relative isolation of the structure being measured. In the equilibrium phase, however, this situation is altered. The dose of RISA has become diluted in the total blood volume and is homogeneously mixed in all the vascular structures seen by the counter. Phantom models of the torcular Herophili are currently being used to determine what fraction the torcular count contributes to the measured equilibrium value. It is expected that the results of the phantom study will enable the blood flow to be stated in absolute cc/min for comparison with nitrous oxide results. Until a number of these phantoms are tested, the results indicate only relative values. For the derivation of the mathematical formula used in calculating blood flow, we refer to the article by MacIntyre, Pritchard, Eckstein and Friedell² for the determination of cardiac output. Others have also reported successful determination of cardiac output by these methods.⁸

RESULTS

Determinations by the radioisotopic method for carotid torcular blood flow were made on 16 patients. On only the last 8, however, was the technique sufficiently standardized for quantitative measurements.

1. Circulation time was measured from the beginning of injection to the highest point of the torcular phase. Among 5 young adults, this measured 5.0 to 6.2 sec; the average was 5.6 sec. The remaining 11 patients had clinical diagnoses of brain tumor (3), cerebral arteriosclerosis (4), subarachnoid hemorrhage (1), internal carotid thrombosis (1), occlusion of anterior choro-

dal artery (1) and traumatic absence of frontal lobe (1). Among these 11 patients the circulation time varied between 51 and 83 sec. The latter occurred in a patient with marked cerebral arteriosclerosis.

2. Characteristics of the dilution curve were analyzed. All patients' curves showed an initial small rise during the first 2 sec. attributable to carotid blood flow. The succeeding elevated curve represented torcular blood flow. In 5 normal young adults this curve occurred between the second second and the twelfth to fourteenth seconds. Among the abnormalities noted in this phase the patient with the prolonged circulation time also exhibited a prolongation of the torcular phase through the eighteenth second; both observations were significant in the face of cerebral arteriosclerosis. One patient with subarachnoid hemorrhage had a prolonged torcular phase through the sixteenth second. One patient with a pineal tumor showed a doubly peaked dilution curve during the torcular phase which in this instance we believe is a manifestation of delayed circulation through the internal cerebral veins and central regions.

3. Quantitative blood flow through the torcular Herophili was calculated in relative and actual values. For purposes of accuracy the elevation of the torcular phase must be near to or exceed 300 counts/sec. Among 5 normal young adults under these conditions the relative values were 210 to 310 E.c./min. As mentioned previously the equilibrium value is currently being studied and hence this unknown L has been left in the relative value.

SUMMARY AND CONCLUSIONS

A radioisotopic method with external counting technique for the determination of blood flow through the torcular Herophili is presented. Radioactive iodinated (131) serum albumin is injected into a common carotid artery and the blood flow recorded by a scintillation counter placed at a specified angle over the torcular Herophili. The theory and procedure of the test are explained. The characteristics of the dilution curve, the carotid torcular circulation time and the relative volumetric results of blood flow are reported in a small group of patients including normal young adults and others with various clinical conditions. Further plans include determination of absolute values and accuracy in a larger number of tests.

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group studied comprised 59 patients selected on the same grounds as for the renal studies

RESULTS AND DISCUSSION

Renal Function Studies Because of its proven accuracy one is well justified to use the creatinine clearance test^{2,3,4} as the starting point of the assessment of the findings. The results of this test have a wide range of normal variation given by most authors as 70 to 130 ml/min or $100 \text{ ml} \pm 30$ per cent per min. In this study only downward variations of 10 per cent or more were regarded as significant (for the sake of brevity the detailed discussion of the reasons for this will be omitted). Nine out of the 50 cases studied were found to show a reduction of function of this magnitude or greater (Table I). It must be noted however that in 3 of these 9 cases although the drop was 15 per cent the post injection values were still considered to be low normal i.e. above 70 ml/min. All of these cases on the other hand exhibited a distinct and significant concomitant rise in the serum creatinine and 2 of the cases also showed a significant decline in phenolsulfonphthalein excretion.

The precise nature of the insult inflicted upon the kidney by the injection of Urokon leading to functional impairment is not known but it is generally assumed to be a chemical trauma. This assumption is supported by the fact that of the 9 cases showing significant changes 6 were examples of occlusive disease in which the impaired outflow allows a higher concentration of the contrast medium to come into contact for a longer period of time with the renal parenchyma than is the case in aneurysms or after the obstruction is removed by grafting. Conversely only 2 out of 17 cases with patent graft and only 1 out of 10 aneurysms showed impairment of function after the injection.

The onset of the damage caused by the contrast dye appeared to be instantaneous and its duration short as a rule. In most instances the renal function returned to normal within 24 to 48 hrs. However in 1 case normal renal function was not restored for 11 days after which a successful operation could be performed with uncomplicated recovery (Case 51). The correspondence of the values of serum creatinine with the results of creatinine clearance was quite close both in the cases listed in Table I showing functional post aortographic impairment and in almost all of the other cases. Thus the serial determination of serum creatinine levels can be used as a simple and accurate means of following the changes in renal function after aortography.

Cardiac Studies The most common symptom after aortography that can be related to the heart was chest pain which was present in about 10 per cent of the patients on whom we performed this examination without general anesthesia. Although this pain is often indistinguishable from angina pectoris apparently in most instances it is not caused by coronary spasm and myocardial ischemia but rather by the flooding by the dye and consequent constriction of the intercostal arteries. The incidence of this symptom among the patients showing electrocardiographic changes was no higher than among the other cases comprising the group.

Pulse and blood pressure changes occurred commonly but without a definite trend and the changes showed no correlation with electrocardiographic alterations.

RENAL AND CARDIAC RESPONSES AFTER TRANSLUMBAR AORTOGRAPHY*

D EVERICK SZILAGYI ROGER T SMITH CLAIBOURNE P SHONNARD AND
HERNAN ALVAREZ

With the current widespread use of translumbar aortography the physiological changes brought about by the introduction of contrast media into the abdominal aorta have assumed the importance of a practical clinical problem. Instances of significant renal and cardiac functional aberrations observed after aortography and the lack of sufficient basic information with respect to the physiologic responses to the intra-aortic injection of radio-opaque substances have prompted us to investigate two aspects of this problem.

METHODS

Technique of Aortography The well standardized technique described by Smith *et al*¹ was employed with some slight modifications. Twenty-five to 35 ml of 70 per cent Urokon was injected manually in 1 to 6 sec. If it was necessary and safe the injection was repeated once or twice.

Renal Function Studies For the investigation of the pre and post injection state of the renal function the 24 hr creatinine clearance^{2,3,4} and the 15 min phenolsulfonphthalein excretion tests were used. Main reliance was on the creatinine clearance test. These tests were chosen for the following practical reasons. They are relatively simple to carry out and yet they have well documented accuracy and reliability; moreover they afford an estimate of both glomerular (creatinine clearance) and tubular (phenolsulfonphthalein excretion) function. The method of creatinine determination was that of Bonsnes and Trussky.² The pre-injection study was performed on the day preceding the aortogram and the post-injection study on the day immediately following. In some cases the clearance was repeated and the serum creatinine was followed serially. The group studied comprised 50 patients of varying clinical background so selected as to provide a representative sample of the class of patients likely to be submitted to aortography.

Cardiac Studies Electrocardiograms were taken the day preceding the aortogram. With the patient in the prone position a preliminary electrocardiogram was obtained just before the injection of the contrast medium. Electrocardiographic tracings were then made immediately after the injection of the dye and five, 10 and 15 minutes later. Records were kept of the pulse rate and blood pressure taken at 5 min intervals. The anesthesia was deliberately varied according to the following scheme: pentothal sodium (25 cases), intraspinal novocaine (18 cases), intraspinal novocaine with nitroglycerine premedication (15 cases). In the last named group 0.6 mg of nitroglycerine was administered sublingually 3 min before the start of the injection. The pre-anesthetic medication was uniform for all groups and consisted of morphine, atropine and barbiturates. The electrocardiographic tracings were obtained through 3 standard limb leads and 3 augmented unipolar leads. The recording apparatus was a Cambridge string galvanometer. The

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Table 2 Electrocardiographic Changes After Translumbar Aortography in 16 Cases Among 59 Studied

CASE NO.	TYPE OF VASCULAR DISEASE	PRE-EXISTING CARDIAC DISEASE	TYPE OF ANESTHESIA	BEFORE INJECTION	ELECTROCARDIOGRAPHIC CHANGES AFTER INJECTION
5	Dissecting Aneur	ASHD with Aneur	Pentothal Sodium	ST depressed in II and III	ST flat in II and III
64 M		ASHD	Spinal	I wave normal	I wave tall in II
9	Leriche			I wave normal	I wave inverted in AVL
59 M	incomplete			I wave normal	I wave tall in II
13	Occluded	0	Spinal	T wave normal	I wave tall in II
57 M	LCI graft	0	Pentothal Sodium	NSR	NSR with nodal premature beats
15	Leriche	0	Pentothal Sodium	ST normal T wave normal	ST depressed in II T wave flat in II
48 M		0	Pentothal Sodium	ST normal	ST depressed in II and III
18				I wave normal	I wave flat in II & AVL inverted in III
59 M	LEI graft	0	Pentothal Sodium	LBBB complete	I RBBB with PVS
19	REI graft			T wave small in I flat in AVL	I wave flat in I inverted in AVI
59 M	REI occlusion	ASHD with hypertension	Pentothal Sodium	AF	AF with PVS
22	Bil CI	ASHD	Pentothal Sodium	T wave diphasic in AVF	I wave inverted in AVF
24	Aneur			NSR QRS 0.07	NSR with nodal premature beats QRS 0.12
55 M	Diffuse AS	ASHD with failure	Pentothal Sodium	T wave normal	T wave inverted and covered in I and III
32	Bil SF	0	Spinal	T wave normal	T wave inverted in II and III
68 F	Occlusion			NSR ST normal	NSR with PVS ST depressed in I
56				T wave normal	I wave inverted in II and III
67 M				NSR	NSR with nodal premature beats
43	RCI	0	Spinal with premed	NSR	NSR with PVS
50 M	Occlusion		Spinal	NSR T wave flat in II slightly inverted in III and AVF	Ventricular bigeminy T wave diphasic in II inverted and covered in III and AVF
44	Abd Aortic	ASHD		NSR	NSR with PVS interpolated
62 M	Aneur	ASHD	Spinal with premed	NSR	
53	LCI and LSF		Pentothal Sodium		
59 M	Occlusion	0	Spinal		
57	RSF Graft				
51 M					
58	Leriche	ASHD with Coronary Occl	Spinal		
55 M		0	Spinal with premed		
61	RCI				
52 M	Occlusion				

Abbreviations L left R right CI common iliac EI external iliac SF superficial femoral AS atherosclerosis ASHD atherosclerotic heart disease NSR normal sinus rhythm LBBB left bundle branch block AF auricular fibrillation PVS premature ventricular systole

Table 1 Changes in Renal Function After Translumbar Aortography in 9 Cases Among 50 Studied

CASE NO	AGE	SEX	SYSTEMIC AS	TYPE OF VASCULAR DISEASE	AMT OF DYE INJECTED	URINALYSIS				CREATININE CLEARANCE ml/min		PER CENT DROP		SERUM CREATININE mg%		15 MIN PSP FACRETION	
						BUN	ALB	SED	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
2	48 M	+		A I Occl Dis	70 ml	15	0	0	N I	146	62	6%		8	17	3%	12%
4	51 M	+		P O gr Fem Occl Dis	90 ml	18	0	0	+	RBC	156	60%		8	19	3%	20%
17	63 M	+		I O gr A I Occl Dis	40 ml	12	0	0	Tr	0	72	45%		6	13	37%	41%
32	60 M	+		I Occl Dis	90 ml	13	0	0	Tr	RBC	191	107		7	13	4%	6%
56	65 F	+		I Occl Dis	105 ml	14	0	0	Tr	RBC	98	23	75%	6	21	28%	12%
38	39 M	+		A I Occl Dis	70 ml	17	0	0	Tr	RBC	168	42	75%	6	21	26%	N I
42	56 M	+		I O gr Abd Aneur	105 ml	12	0	0	0	0	187	104	45%	10	24	16%	16%
47	48 M	+		A I Occl Dis	40 ml	11	0	0	0	0	116	54	55%	10	13	16%	0%
51	41 F	+		A I Occl Dis	80 ml	9	0	0	+++	RBC	122	33	75%	6	23	34%	6%

Abbreviations: A I aorto iliac; I iliac; gr graft; Pre pre aortogram; Post post aortogram; N I no information; AS arteriosclerosis

- 3 Camara A A, *et al*. The twenty-four hours endogenous creatinine clearance as a clinical measure of the functional state of the kidneys. *J Laborat Clin Med* 37:713 1951
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HYPERHYDRATION: A METHOD OF DECREASING THE NEPHROTOXIC EFFECTS OF UROKON AS EMPLOYED IN AORTOGRAPHY*

GEORGE C MORRIS JR, J STANLEY CRAWFORD, ARTHUR C BEALL JR
AND JOHN H MOYER

Renal insufficiency following translumbar aortography has been encountered with increasing frequency during recent years.¹⁻³ A clinical impression was gained by the authors that patients who were given 1 or 2 liters of intravenous fluid immediately prior to aortography did not develop evidence of renal damage. Conversely patients in the usual pre-anesthetic and semi-dehydrated state frequently demonstrate minimal and occasionally marked elevation of the blood urea nitrogen following aortography. The necessity for a satisfactory urinary output in the prophylaxis of renal injury and crystaluria during treatment with certain sulfur derivatives is well accepted. Less well documented is the importance of dehydration in the development of renal insufficiency associated with hemoglobinuria.⁴⁻⁶ With this background 2 groups of dogs were studied to determine the effects of hyperhydration and relative dehydration at the time of intra-aortic injection of nephrotoxic doses of 70 per cent Urokon (R) sodium.

METHOD

Mongrel female dogs were anesthetized with intravenous pentobarbital (30 mg/kg) after hydrating with water through a gastric tube (40 cc/kg). Control determinations of renal function were then made using averages of three 10 min collection periods. Creatinine was used to measure glomerular filtration rate (GFR). Renal blood flow (RBF) was derived from renal plasma flow (RPF) measured by para-aminohippuric acid. The methods and techniques have been described previously.⁶ Later the abdominal aorta was exposed through a retroperitoneal left flank incision. The aorta was occluded below the origin of both renal arteries and 70 per cent Urokon (R) sodium in a dose of 6 cc/kg was injected rapidly through a #18 gauge needle into the aorta above the occluding clamp (Fig 1). After removing the aortic clamp the wound was sutured in layers and the dog given intramuscular

*From the Cora and Webb Mading Department of Surgery and the Department of Pharmacology, Baylor University College of Medicine, Houston, Texas. Supported in part by grant H 1847 from the National Heart Institute of the National Institutes of Health, United States Public Health Service and a research grant from the Houston Heart Association.

Among the 58 cases studied 16 showed electrocardiographic post injection changes that were judged significant (Table 2). The most common finding was inversion of the T wave (in 9 cases) and depression of the ST segment (3 cases). In 6 cases arrhythmia was noted. These comprised mainly premature nodal and premature ventricular systoles. All the changes were instantaneous or at least followed closely the injection of the dye. In 1 instance (Case 5) the depression of ST segment developed gradually over several hours. The alterations were generally transient but in the last named case they persisted for 3 days.

The exact mechanism of the effects of the injection of dye on the heart is not clear. On the whole the electrocardiographic changes can readily be explained by myocardial ischemia most probably resulting from coronary spasm. The obvious mode of production of this spasm would be the irritant action of the dye on the intima of the coronary arteries. There is evidence among our angiograms that the dye injected into the abdominal aorta at the level of the twelfth thoracic or first lumbar vertebra may reflux into the thoracic aorta and indeed into the aortic arch. Thus coronary spasm consequent on intimal irritation by the contrast medium must be regarded as the most probable mechanism underlying the electrocardiographic changes. The reflex systemic arterial constriction seen at times following intra arterial dye injection may add to this response, and the possibility of some noxious effect directly on the myocardium cannot be excluded.

A question of much practical importance is whether pre existing heart disease and the presence of severe distal occlusive disease increase the likelihood of the occurrence of the changes described above. The data at hand are insufficient to justify definite conclusions but they suggest that the answer is in the affirmative.

SUMMARY AND CONCLUSIONS

Before and after the translumbar intra aortic injection of 70 per cent Urokon in 50 patients the changes in renal function were followed by means of 24 hr creatinine clearance, 15 min phenolsulfonphthalein excretion and serial serum creatinine determinations and in 58 patients the alterations in pulse, blood pressure and electrocardiographic tracings were recorded. In 18 per cent of the patients in the first group significant reduction of the renal function was noted. Twenty eight per cent of the patients in the latter group showed electrocardiographic changes mostly those indicative of myocardial ischemia. Both types of functional aberration were instantaneous and virtually always transient as a rule clearing in 48 hrs. In selecting patients for aortography and in timing surgical interventions after aortography the possibility of these physiologic reactions must be kept in mind. Checkup electrocardiographic examinations and serial serum creatinine determinations before and after aortography appear to be obligatory precautionary measures.

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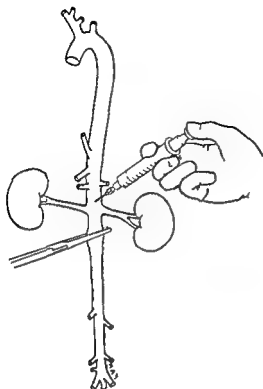


Fig 1 The method of intra aortic injection of Urokon (R). A vascular clamp occludes the aorta distal to the renal arteries to potentiate the quantity of Urokon entering the renal arteries. A retroperitoneal left flank incision offers satisfactory posture.

penicillin. The purpose of aortic occlusion distal to the renal arteries during the injection of Urokon (R) was to promote maximal renal damage from a nonlethal quantity of the drug. Three to 5 days later renal function studies were repeated. Alternate dogs in the series were prepared before intra aortic injection with the following 2 techniques: in 1 group water was withheld for 24 hrs just prior to operation. Dogs in the second group were allowed unrestricted water intake and then given 40 cc/kg of 5 per cent glucose in water intravenously immediately prior to the intra aortic injection. After operation both groups were allowed unrestricted water intake. On completion of the experiment, the animals were sacrificed and the kidneys sectioned for gross and microscopic changes.

Fig 2 A comparison of the glomerular filtration rate, renal blood flow and water excretion following intra aortic injection of Urokon (R). Renal functional impairment is significantly greater in the animals that were dehydrated at the time of injection.

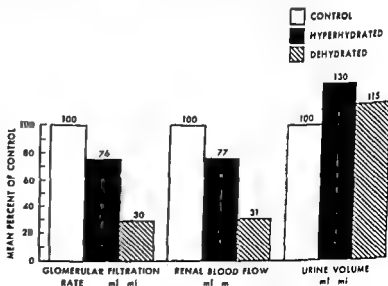


Table 1 Comparison of Renal Functional Impairment Following Intra aortic Injection of Urokon(R) in the Dehydrated State Compared with the Hyperhydrated State

dog	Dehydrated	HEMATOCRIT		GLOMERULAR FILTRATION RATE ml/min		RENAL BLOOD FLOW ml/min	
		C	U	C	U	C	U
1		44	47	28	—	337	66
3		43	43	47	30	337	195
5		36	34	58	32	341	223
7		50	39	87	8	530	53
9		35	31	42	7	298	20
11		42	27	61	8	505	15
13		40	42	31	8	210	45
15		25	26	36	18	136	86
17		43	34	46	42	237	213
19		35	24	37	2	228	35
21		45	23	28	3	147	0
23		39	23	49	8	277	37
Mean		40	34	49	14	293	83
Per cent of control			86		30		31
Hyperhydrated							
2		45	42	63	62	375	338
4		39	39	43	38	179	177
6		40	35	40	45	177	240
8		42	30	38	31	195	166
10		33	29	52	47	264	238
12		26	24	49	23	257	155
14		39	30	43	57	208	240
16		45	36	38	27	244	161
18		39	39	53	51	310	267
20		39	34	35	7	205	55
22		36	36	45	12	203	44
24		31	29	42	17	226	130
Mean		38	34	45	35	237	183
Per cent of control			89		76		77

C—Preoperative control

U—3 to 5 days after injection of Urokon (R)

RESULTS

The results are summarized in Table 1. There was a mean reduction in hematocrit due to sampling and the operative procedure of 14 per cent in the dehydrated group and 11 per cent in the hyperhydrated group. Four dogs in the dehydrated group died before the final renal function studies could be made and are not included in the table. None of the well hydrated animals died. There was a mean reduction in glomerular filtration rate of 70 per cent in the dehydrated group compared to only 24 per cent in the hyperhydrated group. Similarly renal blood flow was reduced 69 per cent in the former and only 23 per cent in the latter (Fig. 2). A severe depression in renal function as represented by a reduction of more than 70 per cent in

both functions was present in 8 of the dehydrated group and in only 2 of the hyperhydrated dogs. There was no significant difference in blood pressure or urine volume between the 2 groups. Grossly, the kidneys of the dehydrated group showed edema and variable diffuse hemorrhagic changes. These changes were minimal in the well hydrated group. Microscopically, in addition to edema and hemorrhage there were many tubules filled with eosin staining material in the dehydrated group. In the hyperhydrated group these changes were minimal or absent.

DISCUSSION

General or spinal anesthesia is commonly employed clinically during aortography usually necessitates withholding oral intake for 10 to 18 hrs. Further because of the brevity of the procedure intravenous fluids are frequently given sparingly or not at all. Thus a hydropenic state is reached at the time of maximal concentration of the radio opaque medium in the kidney. This state of relative dehydration with associated diminished water excretion should offer both an increased concentration and exposure time of the substance in the nephron. Diuresis should reduce these factors.

In this study the dehydrated group of animals showed severe functional damage while the well hydrated group usually had only minimal damage. The study does not suggest the relative importance of dehydration in promoting or hyperhydration in preventing renal damage. However the rate of water excretion was the common denominator for both groups. Hence the magnitude of damage in any given state of hydration may be related to this function.

SUMMARY AND CONCLUSIONS

The nephrotoxic propensity of 70 per cent Urokon as used in translumbal aortography was demonstrated in dogs. Functional renal damage was greatest when water was withheld for 24 hrs. In dogs hydrated sufficiently to produce diuresis minimal damage was usually demonstrated. It is reasonable to assume that diuresis reduced both the concentration and the length of the exposure of this nephrotoxic agent in the kidney.

It is suggested that adequate hydration just prior to aortography may help prevent tragic incidents of renal insufficiency.

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THE VARIOUS EFFECTS OF ANESTHESIA, HYPOTHERMIA AND RENAL ISCHEMIA ON THE KIDNEY IN PATIENTS HAVING SURGICAL THERAPY OF THORACIC ANEURYSMS*

ARTHUR C. BAILL, JR., GEORGE C. MORRIS, JR., MICHAEL F. DE BAKKY
AND JOHN H. MOYER

Surgical excision has now been established as the method of choice in the treatment of aneurysms of the aorta. Because hypothermia has been found to provide a protective effect against ischemic damage to the spinal cord^{1,2} it has been frequently employed in the excision of certain types of aneurysm of the thoracic aorta.³ The purpose of this study was to determine the various effects on the kidney of the combination of anesthesia, hypothermia and temporary aortic occlusion.

METHOD

Eleven male and 3 female patients ranging in age from 44 to 64 years were studied. All had fusiform aneurysms involving the upper segment of the descending thoracic aorta and were treated by resection with homograft replacement. Observations were made of mean blood pressure, glomerular filtration rate, renal blood flow, and water and electrolyte excretion. Mean blood pressure was derived from auscultatory systolic and diastolic pressures by adding one third of the pulse pressure to the diastolic pressure. Glomerular filtration rate and renal blood flow were determined by the use of inulin and paraaminohippurate. Sodium and potassium determinations were made using a Beckman flame photometer. Methods and techniques have been described previously.⁴ Three 10 min. collection periods were obtained before the patients were anesthetized and two 10 min. collection periods immediately after anesthetization. Hypothermia was then induced using an electrically controlled water-cooled blanket. Maximum reduction in temperature ranged from 29° to 33° C. Observations consisting of two 10 min. collection periods were made at variable periods somewhat above maximum hypothermia and at the point of maximum reduction in temperature. These were repeated after blood pressure had become stabilized following release of aortic occlusion and again after rewarming. Finally, three 10 min. collection periods were obtained 7 to 11 days after operation. All observations were then subjected to statistical analysis and compared with control values.[†] Two patients were excluded from the tables as explained below.

RESULTS

The data obtained from these observations on the effects of anesthesia, hypothermia and aortic occlusion on renal hemodynamics and water and electrolyte excretion are presented in Table 1. These are represented diagrammatically in Figure 1 which reveal that mean blood pressure was reduced slightly during anesthesia, hypothermia and following the period of aortic occlusion. These changes, however, are not significant. With the induction of anesthesia there was minimal depression of glomerular filtration rate and

*From the Cora and Webb Mading, Department of Surgery and the Department of Pharmacology, Baylor University College of Medicine, Houston, Tex.

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both functions was present in 8 of the dehydrated group and in only 2 of the hyperhydrated dogs. There was no significant difference in blood pressure or urine volume between the 2 groups. Grossly the kidneys of the dehydrated group showed edema and variable diffuse hemorrhagic changes. These changes were minimal in the well hydrated group. Microscopically in addition to edema and hemorrhage there were many tubules filled with eosin staining material in the dehydrated group. In the hyperhydrated group these changes were minimal or absent.

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†Statistical analyses by R. A. Seibert, M.D.

Table 1 Effects of Anesthesia Hypothermia, and Renal Ischemia on Renal Hemodynamics and Water Electrolyte Excretion

PATIENT	MEAN BLOOD PRESSURE mm Hg				GLOMERULAR FILTRATION RATE cc/min				RENAL BLOOD FLOW cc/min				DURATION OF AORTIC OCCLUSION TIME IN MIN									
	C	A	D ₁	D ₂	C	A	D ₁	D ₂	C	A	D ₁	D ₂	D ₃	D ₄	D ₅							
H B	99	99	94	102	95	89	98	86	78	81	64	46	47	99	868	748	568	323	709	560	1031	64
J A	99	72	75	77	76	92	87	100	99	71	65	49	96	115	1459	1378	1418	991	629	1042	2259	37
M P	70	70	70	77	—	80	—	88	59	50	50	—	34	—	772	380	435	442	—	304	—	47
P G	73	70	77	77	—	—	75	101	81	70	71	—	—	118	1065	1131	673	631	—	—	1163	63
P S	77	77	52	73	85	92	93	82	102	84	98	51	64	112	1006	1385	1261	1067	785	625	973	41
J D	103	97	—	87	99	100	101	101	89	—	72	38	104	100	1411	1215	—	651	669	757	810	35
C S	132	127	125	120	125	130	—	97	81	49	46	58	74	—	802	846	354	392	568	697	—	40
W K.	120	117	105	85	93	125	125	109	157	85	55	25	85	85	993	1256	948	631	545	705	866	53
S R	115	96	87	74	81	100	104	111	37	78	71	54	121	115	825	338	558	666	816	1064	1078	33
D T	82	97	86	112	100	81	113	32	38	63	41	31	27	50	485	579	592	463	522	383	424	47
Mean	97	92	86	89	94	100	100	91	82	70	63	44	72	99	969	926	756	626	655	682	1075	
Mean % of Control		95	90	94	93	102	105		93	88	75	54	79	115		95	82	66	73	73	105	
P Value		0.50	0.30	0.40	0.50	0.50	0.50		0.50	0.05	0.01	0.001	0.20	0.50		0.50	0.20	0.05	0.05	0.05	0.50	

[illegible]

Key to abbreviations

C=Control observations (average of three 10 min periods)

¹⁸—Observations after the induction of anaesthesia (average of two 10 min periods)

D = Observations after initial reduction in body temperature (average of two 10 min period)

D = Observed after maximum reduction in body temperature (average of two 10 min period)

D = Observations after release of zoster exclusion (average of two 10 min periods)

D = Observations after rewarming (average of two 10 min periods)

D = Observations / 4 days after operation (average of three 10 min periods)

$$\text{Methylation} = 1 \times$$

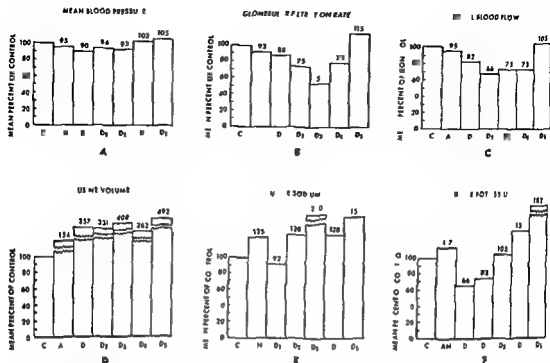


Fig 1 Diagrammatic representation of the effects of anesthesia hypothermia and renal ischemia on renal hemodynamics and water and electrolyte excretion

renal blood flow associated with a slight increase in the urinary excretion of water sodium and potassium. These changes are also not significant.

As body temperature was reduced glomerular filtration rate fell progressively to 75 per cent of control values (Fig 1B). Following the period of aortic occlusion which varied from 33 to 64 min, there was further depression of glomerular filtration rate to 54 per cent of control values. However, by the time the normothermic state was reached, glomerular filtration rate had returned toward normal and within 7 to 14 days it had reached control values.

The renal blood flow paralleled the fall in glomerular filtration rate, reaching 66 per cent of control values during maximum hypothermia (Fig 1C). Following release of aortic occlusion, there was immediate return to pre-occlusion levels with no further change on rewarming. At the end of 1 to 2 weeks, normal values were obtained.

Rather than the usual depression of urine volume which is associated with a fall in glomerular filtration rate under normothermic conditions, urine volume increased with reduction of body temperature (Fig 1D). Following the period of aortic occlusion, urine volume was further elevated. The changes in sodium excretion (Fig 1E) closely paralleled those of urine volume.

In contrast to the rise in water and sodium excretion associated with hypothermia, there was depression of potassium excretion, and this was greatly increased after return to normothermic conditions (Fig 1F).

With anesthesia, hypothermia, and aortic occlusion, there were no significant changes in the hematocrit. Plasma potassium levels were depressed slightly during the period of maximum hypothermia, as were plasma sodium levels 1 to 2 weeks after operation. During the period of aortic occlusion, there was no measurable renal function.

DISCUSSION

Contrary to the generally held belief that anesthesia is associated with depression of renal function⁶ minimal changes were noted in the patients studied. This was perhaps related to the fact that light anesthesia associated with muscular relaxing agents was used. However, because of the diversity of anesthetic agents employed and the small number of patients the significance of this is not known.

The changes in renal hemodynamics, water and electrolyte excretion during hypothermia and following temporary occlusion of the thoracic aorta appear similar to those seen in the laboratory animal.^{7,8} The reduction in glomerular filtration rate and renal blood flow associated with a reduction in body temperature appeared related to the hypothermic state. The lack of fall in urine volume and sodium excretion associated with this depression of glomerular filtration rate was probably related to a depression of tubular function during hypothermia. Additional evidence of depression in tubular function during hypothermia is demonstrated by a fall in urinary potassium excretion in active process of the tubules.

Following the period of aortic occlusion, the further fall in glomerular filtration rate as well as the rise in urine volume and sodium excretion was probably related to transient glomerular and tubular depression associated with renal ischemia. This ischemia did not appear to influence renal blood flow, and its overall effect appeared to be temporary as return toward normal had occurred as the normothermic state was reached. The depression of glomerular filtration rate and renal blood flow remaining after rewarming was not dissimilar to that seen in the laboratory immediately following hypothermia in the dog.⁸

Two of the patients studied were not included in this presentation, as renal artery occlusion unilateral in one and bilateral in the other was necessary as well as aortic occlusion. Although some protection from ischemic renal damage by hypothermia can be demonstrated in the laboratory,^{8,9} both of these patients showed severe damage following the procedure.

SUMMARY

The effects of anesthesia, hypothermia and renal ischemia on renal hemodynamics, water and electrolyte excretion in 14 patients having excisional therapy of thoracic aneurysms have been presented. No significant changes were seen following the induction of anesthesia. Reduction in body temperature was associated with a progressive fall in glomerular filtration rate and renal blood flow. This was not accompanied by significant alteration in urine volume or sodium excretion but by a depression of potassium excretion. Temporary renal ischemia from occlusion of the thoracic aorta produced a further depression of glomerular filtration rate with a significant rise in urine volume and sodium excretion. These effects were transient, however, and return toward normal occurred with rewarming. Renal function had returned to normal when final observations were made 7 to 14 days after operation.

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EVALUATION OF ARTERIAL HOMOGRAFTS FOLLOWING SIX YEARS OF IMPLANTATION IN DOGS*

EDWARD B C KEEFER AND FRANK GLENN

Knowledge concerning the ultimate fate of an homologous arterial implant in the human should influence considerably the trend in vessel surgery. The prosthesis best suited for arterial replacement even after many years of research is still a matter of contention and open to further investigation. The outstanding reports of human vascular surgery in the last 8 years credit the dog as being much more than man's best friend. We are all aware that it is difficult to predict human end results from animal experiments. Statistically the dog lives from 8 to 12 yr and man from 60 to 80 yr. Therefore the authors wish to assume that a 6 yr implantation period in the dog is the equivalent of 30 to 50 years in the human. In 1952 the authors presented a report to the Forum on a 2 yr observation of a series of dogs with thoracic aorta homografts.³ Additional and more significant data following a 6 yr study is presented here.

PROCEDURE

In 1950 a litter of 6 puppies (age 3 mos wt 10 to 17 lbs) had an adult dog arterial graft of approximately the same caliber as the host's artery implanted into the thoracic aorta. The homograft was inserted by an end to end anastomosis using a continuous everting mattress suture of 6/0 chromic cat gut on a minimal trauma needle. The grafts had been stored for varying periods of time in nutrient media and refrigerated at 0 to 4°C according to

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Fig. 1 Dog #1 Photomicrograph magnification X 38. Ossification of graft at proximal suture line. Graft to right side of picture.

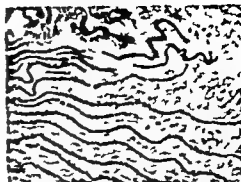


Fig. 2 Dog #1 Photomicrograph magnification X 180. Calcified elastic fibers of homograft.

the method then used by the New York Blood Vessel Bank and described by Gross *et al*¹ and Keefer *et al*².

Two years later an exploratory thoracotomy was performed on all 6 dogs. Postoperative adhesions made it difficult to measure the outside diameter of the vessel. These measurements have been presented in Table 2 of the previously reported 2 yr study.³ At this time angiography was employed to visualize the grafts' caliber.

Six years after the initial operation the dogs weighed from 41 to 60 lbs and once more were explored by thoracotomy. The pleural adhesions resulting from the previous surgery made it impossible to obtain accurate measurements of the implant; however the grafts were seen and palpated. The lumen of these homografts were opacified by retrograde carotid arteriography.

One dog died of unrelated causes 4 yr postoperatively. The other 5 are alive and well.

RESULTS

Owing to the technical difficulties resulting from healing, scarring and adhesions, it was not possible to make accurate measurements of the outside caliber of the homograft suture line and adjacent host's vessel wall. Nevertheless, our observations suggested that no real growth of the homograft or suture line occurred. It is conceivable that some dilatation may have been present in these areas relatively soon after implantation. However, following

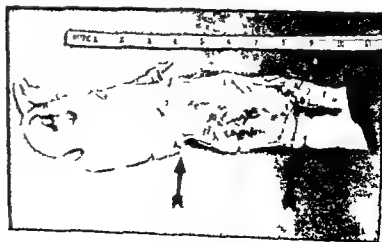


Fig. 3 Dog #3. Gross autopsy specimen 4 yr after implantation. Initial surface smooth (cracks due to brittleness and opening of vessel). Arrows point to suture lines.



Fig. 4 Dog #3 Photomicrograph magnification $\times 20$ Chromic catgut suture material

prolonged implantation, the calcification and ossification of the homograft precluded any increase in size

No rupture, incursion or stricture formation has been observed in this series. These homografts have continued to function as satisfactory prostheses withstanding the normal rigors of a dog's life, such as repeated pregnancies, fights, etc.

Histological section of the thoracic aorta of dog #5 with a 1 yr arterial implant showed several pathologic changes. No atheroma or atheromatous plaques in the intima were produced. The intima was relatively acellular and thickened over the graft and suture lines. An occasional small fleck of calcification could be seen in the intima of the graft. The media and adventitia of the graft and the immediate adjacent host's vessel wall revealed exten-

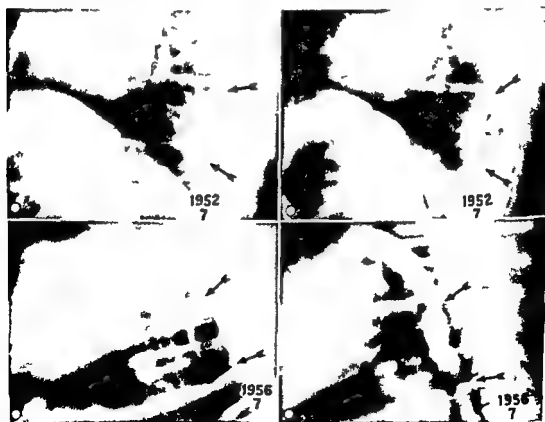


Fig. 5 Dog #7 Left side—Chest x ray
Half moon ossification of graft between
arrows not an aneurysm

Right side—Arteriography

Fig 6 Dog #7 Operating photo graph 19. White ossified graft not an aneurysm but due to larger donor graft



sive calcification with several large areas of ossification (Fig 1). The elastic fibers were impregnated with calcium (Fig 2). This medial calcification and bone formation of the homograft was observed to extend for a short distance into the host's vessel wall (Fig 3). The so-called absorbable chromic catgut suture was found at the suture line 6 yr postoperatively (Fig 4). The vessel lumen was not encroached upon except for minimal small mural thrombi in some areas (Fig 5).

After 2 yr of implantation dog #7 was thought to have formed a fusiform aneurysm of the graft. It is now apparent that this dilatation is a bony thickening in the graft wall (Fig 5 & 6). The original anastomosis is responsible for the slight narrowing at the distal suture line and the palpable thrill over the host's distal artery noted during the 1956 thoracotomy. All the grafts

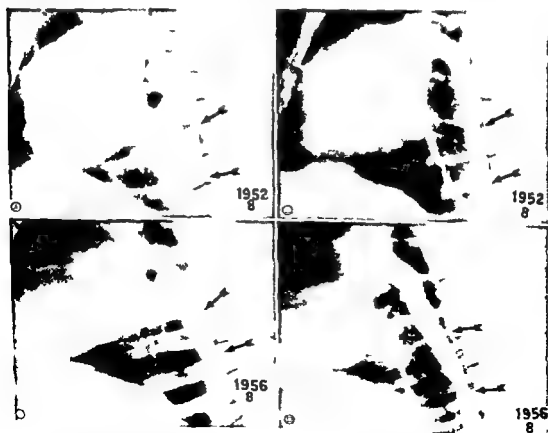


Fig 7 Dog #8 Left side—Chest X ray
Graft calcified between arrows

Right side—Arteriography

were palpated as hard tubes and no appreciable narrowing or dilation was observed

Chest roentgenograms showed opaque calcified homografts in all dogs (Fig 7) Arteriography envisaged a patent lumen with no stricture or obstruction to the blood flow (Figs 5 & 7)

This study will be continued as long as these animals survive

CONCLUSION

After 6 years of implantation the degree of calcification and bone formation observed in the homograft has not impaired the function as a blood conduit The degenerative processes which developed in the arterial grafts studied in no way resembled human atherosclerosis as no atheroma were found and calcification of the elastic fibers occurred uniformly throughout the media and adventitia with some areas of actual bone formation In these dogs irrespective of the questionable absorbable suture material no demonstrable growth of the suture line was observed The homograft has not grown with the growth of the animal The degenerative process described above suggests that extreme care to prevent trauma and mural thrombus production on the intimal surface is an important consideration in arterial segment replacement

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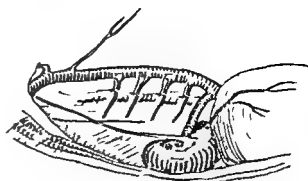
THE PROGRESSION OF EXPERIMENTAL ATHEROSCLEROSIS AFTER LUMBAR SYMPATHECTOMY*

THOMAS O MURPHY JOHN J HAGLIN AND DAVITT A FELDER

The initial salutary effect of lumbar sympathectomy in clinical atherosclerosis is well documented^{1,2} and has made this a popular procedure The later or secondary effects of this procedure in atherosclerotic individuals has not yet been clearly determined In spite of the intensive studies upon atherogenesis over the past decade there have been no reports on the effect of an autonomic denervation upon this process This is a report of an empirical approach to this problem in an experimental animal the rabbit

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Fig. 1 Lumbar sympathetic ganglia in the rabbit



METHOD

Animals were made atherosclerotic using the technique of Wicker and Hueck.² A rabbit pellet chow was prepared by the following formula:^{*}

Dehydrated alfalfa meal	985 lbs
Stabilized animal fat	15 lbs
Cholesterol	2 lbs
Toasted soya flour	118 lbs

The animal fat was stabilized by adding Tennox II.[†] Rabbits from 5 to 7 lbs were used which consumed from 50 to 150 gm of feed daily.

A bilateral lumbar sympathectomy was carried out in the rabbit using intravenous nembutal anesthesia. A midline abdominal incision allowed the sympathetic ganglia and trunks to be identified as thin white fibers in the retro-aortic area (Fig. 1). The trunks and ganglia (L_1 to L_4) were removed from the level of the renal arteries to the pelvis and a peri-aortic stripping of all adventitia performed to assure an adequate sympathectomy. In other sham operations the abdomen of the animal was explored and the aorta manipulated with forceps in a much more traumatic fashion than was done during the course of a sympathectomy but with care taken not to damage or sever the sympathetic trunks.

The animals were divided into 6 groups as follows:

1 Twelve animals were placed in cages in the laboratory and allowed to eat a normal rabbit pellet chow of similar constitution as noted but without the addition of animal fat or cholesterol. These animals were not operated upon during the course of the experiment but were followed to evaluate the normal aging process of the rabbit aorta.

2 Twelve animals were operated upon having a sham operation and aorta manipulation. These were then placed on the normal diet as in group 1.

3 Twelve animals were operated upon having a bilateral lumbar sympathectomy and peri-aortic stripping below the renal arteries. These were fed a normal diet.

4 Twelve animals were started upon an atherogenic diet without surgery of any sort.

5 Twenty-four animals had a sham operation with an aortic manipulation and fed an atherogenic diet.

^{*}Prepared by Archer Daniels Midland Company Minneapolis, Minnesota

[†]Produced by Tennessee Eastman Corporation

6 Twenty four animals had bilateral lumbar sympathectomies and periaortic stripping below the renal arteries. Upon recovery they were fed the atherogenic diet.

The animals were followed on these diets from 6 mo to 1 yr to evaluate the late effect of the operative procedures. The only additive to the diets other than the above was the occasional feeding of fresh greens to the animals.

RESULTS

The animals were followed for the development of cholesteremia and the serum cholesterol measured after the method of Bloor.⁴ Within 3 wk the serum of the animals on the atherogenic diet showed a marked lipemia. Serum cholesterol levels in the animals eating a normal diet ran between 18.3 mg per cent to 100.2 mg per cent. The animals which were fed the atherogenic diet ranged between 583 mg per cent to 1020 mg per cent blood cholesterol.

Serum Cholesterol Determinations

GROUP	MINIMUM	MAXIMUM	MEAN
1	48.3 mg %	100.2 mg %	72.9 mg %
2	50.3 mg %	84.2 mg %	76.3 mg %
3	58.3 mg %	90.3 mg %	72.4 mg %
4	583 mg %	2373 mg %	1242 mg %
5	982 mg %	1540 mg %	1312 mg %
6	613 mg %	4020 mg %	1320 mg %

Animals were kept on their respective diets for 6 mo to 1 yr prior to sacrifice to assure that the lesions which developed would be beyond the acute stage. The animals were sacrificed and the entire heart, aorta and femoral arteries were removed and the vessels opened. Tissue samples were taken for microscopic examination from the ear, fore leg and hind leg.

Achieving an accurate objective evaluation of the degree of degenerative changes in the arterial bed is difficult. The rabbit aorta is small and the degree of involvement is a relative thing. In this series we have preferred to grade the progression of the disease from zero to four as follows:

Grade zero No atherosclerosis

Grade 1 Very little thickening of the intima without formation of true atherosclerotic plaques (microscopic evidence of the disease)

Grade 2 Occasional atherosclerotic plaques

Grade 3 Many atherosclerotic plaques with coalescence in many areas

Grade 4 Almost solid coalescence of the plaques in the aorta or evidence of occlusion of a major artery due to degenerative changes

Groups 1, 2, 3 Animals in groups 1, 2, 3 which were fed a normal rabbit chow showed no signs of atherosclerosis even when kept in the laboratory for a 2 yr period. All animals showed grade zero atherosclerosis.

Group 4 animals (normal animals on the atherogenic diet) showed various degrees of atherosclerosis of the aorta above the renal arteries but with little or no evidence of the disease below this level. Microscopic section of the central artery of the ear showed the typical atherosclerotic lesion; however, the

*The Mean Degree of Atherosclerosis of the Aorta
and Peripheral Vessels*

GROUP	THORACIC AORTA	LUMBAR AORTA	PERIPHERAL VESSELS
1	0	0	0
2	0	0	0
3	0	0	0
4	2-3	1-2	0
5	2-3	2	0
6	2-3	1	2-3

smaller arteries and arterioles of the extremities showed only rare lesions. The mean involvement of the group was graded 1-2 to 3 above the renal arteries and 1 to 2 below this level.

Group 5 animals (sham operation with an atherogenic diet) showed similar atherosclerotic degeneration of the aorta and main arterial branches above the renal arteries. Below the renal arteries there was a slight increase in the degree of atherosclerosis in the area of the trunk to the aorta. There was no change in the peripheral vessels. The mean involvement of the group was grade 2 to 3 above the renal arteries and 2 below the renal arteries.

Group 6 animals (sympathectomy and atherogenic diet) showed severe atherosclerotic degeneration above and below the renal arteries with extensive atherosclerotic degeneration involving the superficial femoral arteries as far as they could be examined (Fig. 4). In 2 of the 21 animals the dissection of the terminal aorta was enough to cause thrombosis of this area.

Microscopic tissue section showed numerous areas of small vessel involvement in the thigh sections but only rare involvement of the biopsy from a comparable area in the foreleg.



Fig. 2 The lumbar aorta showing the atherosclerotic plaques stained with carlot red (Sudan IV) stain. (A) Normal aorta. (B) Atherogenic diet without surgery. (C, D) Atherogenic diet with lumbar sympathectomy.

DISCUSSION

The experiments of Taylor, Baldwin and Hass and of Baldwin *et al*⁶ have shown that areas of arterial injury are predisposed to the formation of atherosclerosis in hypercholesterolemic rabbits. The normal reparative process of a damaged artery was the formation of a new arterial wall within a proliferating intima. When such damage was associated with hypercholesterolemia there was a localized deposition of lipids and cholesterol with formation of foam cells.

In view of these data to evaluate only the effect of the sympathectomy it was necessary to carry out the sham operation with manipulation and traumatization of the aorta in a manner similar to that done during the sympathectomy and peri-aortic stripping. The finding of atherosclerotic changes in the peripheral arteries of the extremities was rare in the control animals. However, in the sympathectomized animals such changes were noted with frequency. We may conclude that the more advanced degenerative changes of atherosclerosis in the aorta and peripheral vessels is somehow related to the sympathectomy.

The cause and effect relationship in this experiment is not clear. There is evidence that the changes of physical stresses upon the arterial wall was related to the progression of atherosclerosis. It has been demonstrated that following sympathetic ganglionectomy there is a lessening of the peripheral resistance in the area affected and thus a higher rate of blood flow into this area. Furthermore, we may speculate that there may be a weakening of the muscular wall of the artery due to paralysis of the smooth muscle fibers. It is our contention that these 2 factors contribute to the increase in degeneration found in the sympathectomized animals.

SUMMARY AND CONCLUSIONS

A comparison of the degree of atherogenic degeneration of the rabbit aorta and peripheral arteries was made between normal and sympathectomized animals. It seems conclusive that trauma to the abdominal aorta increases the degree of atherosclerosis and further that a sympathectomy of the arterial bed causes an even greater degree of atheromatous degeneration in both the aorta and the distal arteries of the extremities.

It is difficult to correlate these findings with human atherosclerosis. However, it is suggested that these data may cause one to re-evaluate the indications for sympathectomy in a patient who has a disturbed lipid metabolism.

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CENTRAL AND DIFFERENTIAL HYPOTHERMIA (INTRAPERITONEAL) IN PREVENTION OF ISCHEMIC SHOCK AND PARAPLEGIA FOLLOWING TEMPORARY OCCLUSION OF THE THORACIC AORTA*

WILLIAM M. PARKINS, MAX BEN AND HARRY M. VARS

The data we wish to report at this time are representative of one aspect of a continuing study designed to (1) more clearly define the relative roles of the liver and intestinal tract in the pathogenesis of ischemic shock and to (2) determine the efficacy of generalized and differential hypothermia in combating the irreparable tissue damage induced by general and regional occlusions of the circulation.

This presentation will be confined to current results from the use of hypothermia induced intraperitoneally and of chlorpromazine in preventing ischemic shock and paraplegia which follows temporary occlusion of the thoracic aorta.

METHOD

Healthy adult dogs were anesthetized with pentobarbital (25 mg/kg) intravenously supplemented by additional nembutal as required. In the chlorpromazine group 5 mg/kg was used. Three mg/kg IV was followed by one half the usual amount of nembutal required for anesthesia. In addition 1 mg/kg chlorpromazine was given 1 hr after the initial dose and 1 mg/kg was repeated just prior to aortic occlusion.

Techniques of placement of catheters for blood sampling, blood pressure and the positioning of a specially designed balloon catheter for occlusion between the 8th and 10th intervertebral space were previously described.¹

The intraperitoneal method of differential cooling previously reported² involved the recirculation of ice cold saline through the opened abdomen during the period of occlusion. The cooling procedure has been simplified in the present experiments by the single rapid intraperitoneal injection of iced saline (65 cc/kg) supplemented by an ice pack over the lower abdomen. When the cold saline (0 to 5°C) was administered within 2 min after the aortic occlusion a differential cooling persisted with the intestine being cooled to about 20 to 26°C while the oral temperature was reduced to a range of 30 to 32°C. Without obstruction of splanchnic blood flow the cold was equilibrated throughout the body and within 1 hr a generalized hypothermia to about 25°C was obtained. The hypothermic animals were rewarmed to about 35°C within 2 to 3 hr by forced air heat under and around the body supplemented by a heat bulb over the abdomen.

RESULTS

Occlusion of the thoracic aorta at T 8 to 10 reduced the arterial pressure distal to the obstruction to an average level of 16 mm Hg. The tolerance of the normothermic controls to 30, 60 and 120 min of occlusion (7.8 to 10) mm shown in Table 1. The incidence of paraplegia due to spinal cord damage

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Table 1 Temporary Occlusion of the Thoracic Aorta (T8-10) in Normothermic Control Dogs

OCCLUSION TIME (MINUTES)	NUMBER OF DOGS	SURVIVING HRS AFTER RELEASE OF OCCLUSION		NUMBER PARAPLEGIC
		24	72	
30	10	8	8	4
60	10	4	4	4
120	10	0	0	—

Table 2 Temporary Occlusion of the Thoracic Aorta (T8-10) in Hypothermic Dogs With and Without Chlorpromazine

OCCLUSION TIME (MINUTES)	METHOD OF INTRA- PERITONEAL COOLING	NUMBER OF DOGS	SURVIVING HRS AFTER RELEASE OF OCCLUSION		NUMBER PARAPLEGIC
			24	72	
60	Differential	5	5	5	0
120	Differential	5	5	5	3
120	Generalized	10	7	7	0
120	Generalized + chlorpromazine	10	8	8	0

and the mortality from ischemic shock is evidently a function of the duration of the reduced blood flow and oxygen supply. The degree and duration of the post occlusion hypotension and the extent of the progressive hemoconcentration which followed was increased after the more prolonged occlusion. The most severe mucosal sloughing and hemorrhage into the lumen of the intestine and bloody mucous discharge from the rectum was consistently observed in the 1 and 2 hr normothermic animals which died within 2 to 6 hr after occlusion.

The efficacy of differential hypothermia as compared with generalized precooling with and without chlorpromazine is shown in Table 2. By the intraperitoneal injection of 0 to 5°C saline immediately after occlusion of the thoracic aorta a greater reduction in temperature of the intestine was obtained, and all animals survived an interval of ischemia which was invariably fatal to normothermic dogs. About half of the differentially cooled animals which survived 1 and 2 hr of occlusion were paralyzed. In the generally pre-cooled group the temperature of the blood and presumably the spinal cord was below 28°C and no evident paraplegia occurred. In the differentially cooled dogs the hypothermia induced after occlusion was localized and afforded inadequate protection for the spinal cord in the first hour of ischemia.

The protection against shock and death was somewhat less effective in the pre-cooled dogs since 5 of the 20 pre-cooled did not survive. No significant difference was observed between the 10 pre-cooled dogs which received chlorpromazine and the 10 dogs without chlorpromazine except as to the nebulant requirements for anesthesia. Bloody diarrhea and mucosal sloughing was minimal and the post release hypotension and hemoconcentration were much less severe in the surviving dogs of both hypothermic groups.

DISCUSSION

Pontius and associates² found hypothermia induced by surface cooling to be highly effective in preventing the paraplegia resulting from cord damage due to high level occlusion of the thoracic aorta but was of little influence upon the mortality. Essentially the same result was reported in our published experiments in which generalized precooling to 30°C was induced by external surface cooling or by internal blood cooling.¹ However, when the rectal temperature was reduced to about 25°C by blood cooling 7 of 11 dogs survived the 1 hr occlusion without paralysis. Burch *et al*³ report survival of dogs after 60 min of occlusion of the thoracic aorta in which the liver was cooled by coils placed about the liver. Raffucci *et al*⁴ were able to increase the tolerance time of dogs to temporary ligation of the afferent circulation of the liver with generalized hypothermia induced by surface cooling. We reported last year our negative results with liver arterialization and the moderate degree of protection against the ischemic shock obtained with differential hepatic hypothermia.

Though the tolerance time of the spinal cord to occlusion of the thoracic aorta has been markedly increased by various methods of generalized hypothermia a far greater degree of protection against the ischemic shock has resulted from intraperitoneal precooling and especially the rapid differential cooling of the intestine by cold saline.

SUMMARY AND CONCLUSION

Both the method and degree of cooling affect significantly the protection afforded by hypothermia in the ischemic shock and paraplegia resulting from occlusion of the thoracic aorta. Thus generalized hypothermia to 30°C or lower induced prior to occlusion by surface, blood or intraperitoneal cooling though highly effective in preventing paraplegia may have little effect upon the shock syndrome and mortality.

Generalized precooling by the intraperitoneal administration of iced saline greatly increased the tolerance time to occlusion as judged by the 15 of 20 dogs which survived 2 hr of ischemia without paralysis. Differential cooling of the viscera by rapid injection of 0 to 5°C saline intraperitoneally just following occlusion was the most efficacious in reducing symptoms and mortality in this type of ischemic shock. Post-occlusion hypotension, hemoconcentration, bloody diarrhea and sloughing of the intestinal mucosa are minimal or absent and dogs survived a 2 hr interval of occlusion which was invariably fatal to normothermic controls.

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VASCULAR CHANGES AND DIRECT TISSUE EFFECTS IN SEVERE COLD INJURY*

W L OGILVY M A ENTIN AND D R WEBSTER

This investigation was undertaken to observe and assess the effect of cold directly on tissue and the vascular changes that occur on exposure to cold and thawing

In our experiments, the dog was the animal used and the part subjected to the cold injury was the thigh

The investigation was divided into 2 experiments but the animals were exposed to the same degree of cold and for the same duration throughout the whole investigation In the first experiment an attempt was made to evaluate the physiopathological changes in cold injury by means of a skin grafting procedure and in the second experiment an assessment of blood flow in the cold injured area was made by means of radio active phosphorus

METHOD

Experiment 1 Twenty dogs were used in this part of the investigation Intravenous nembutal was the anesthetic used throughout

When anesthetized the dog had one hind leg and the corresponding side of the body clipped and shaved A split thickness strip of skin was then removed from the dog's side and this strip of skin was divided into four sections A, A1, B and C The leg with the foot protected by an impervious dressing was then immersed in a tank containing 95 per cent ethyl alcohol and CO₂ ice at -20°C for 1 hr This allowed only an area of the thigh to be exposed to the freezing mixture

The 4 sections of skin were disposed as follows A and A1 were kept at room temperature and B and C were placed in separate rubber gloves the cuffs tied securely and immersed in the alcohol ice mixture along with the leg

After 1 hr immersion a split thickness section of skin section D was removed from the lateral aspect of the thigh Skin section B was thawed by immersion in water at 12°C for 5 min, and section C was allowed to thaw at room temperature

The 5 sections of skin A A1 B C and D were then re applied as autografts and the 5 areas on the dog's side and leg then represented the following Area A—Skin frozen *in situ* (section D) then excised and re applied to a normal bed Area A1—Normal skin (section A1) on a normal bed This was a control normal skin graft Area B—Excised skin (section B) frozen then rapidly rewarmed and re applied to a normal bed Area C—Excised skin (section C) frozen then slowly rewarmed and re applied to a normal bed Area D—Normal skin (section A) on a damaged bed (Fig 1)

RESULTS

The survival of the skin grafts was assessed clinically after 11 days During this time however 6 of the dogs had to be excluded from the series because

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Fig 1 The side and thigh of dog 49 showing the distribution of the five sections of the skin after they have been sutured into the designated areas (see text)

of technical difficulties or death. This left 14 dogs for assessment and the skin graft survivals were as follows: Area A—86 per cent; Area A1—90 per cent; Area B—0 per cent; Area C—28 per cent; Area D—7 per cent.

The grafted areas were also biopsied 14 days after the cold injury and although the histological sections showed the structural changes present at this time they did not contribute any information as to why some grafts survived and others did not.

Experiment 2. The dogs were anesthetized with intravenous nembutal; then both hind legs were clipped and shaved. One leg with the foot protected was then immersed in the alcohol-ice mixture at -20°C for 1 hr, following which it was allowed to thaw at room temperature. The following day 10 cc of blood were removed from the dog and incubated with 100 microcuries of radio-active phosphorus at 37°C for 30 min. This blood was then re-injected into the dog and P^{32} activity in the injured leg and normal leg was recorded. Readings were taken at 5, 15, 30 and 60 min following injection and daily thereafter until any sloughing of skin occurred, which was usually around the fourth day. At the end of 14 days the total area of tissue loss was measured on the cold injured limb.

RESULTS

Sixteen dogs were used in this part of the investigation, 3 of which had to be excluded from the series. Recordings of P^{32} activity in the control and injured legs were made in the remaining 13 dogs.

The area of tissue loss after 14 days was very variable, the variation being from 9.76 to 43.97 square inches.

It was found that the greater the initial difference in P^{32} activity between the control and cold injured limbs, the greater was the subsequent tissue loss after a 14 day period (Fig 2 A and B).

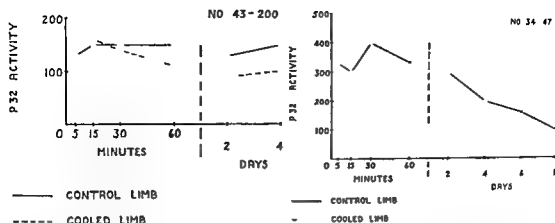


Fig 2 A The graph of P^{32} activity in the dog in which the final loss of tissue in the cooled limb was 11.07 square inches at the end of 14 days

Fig 2 B The graph of P^{32} activity in the dog which showed an extensive loss of skin at the end of 14 days (43.97 square inches)

DISCUSSION

The technique used in the skin grafting procedure was apparently adequate since the normal skin graft control (area A1) showed a 90 per cent survival rate.

The skin graft on areas A and D may be considered together. The skin applied to area A had previously been frozen *in situ* and survived in 86 per cent of cases even when the skin remaining on the cold injured limb became necrotic. Although showing some histologic structural changes which were presumably due to the direct action of cold, it is felt that these survived because they were saved from the vascular sequelae of edema, stasis, ischemia and thrombosis which occur during and after thawing.

The skin applied to area D was normal skin and it survived only partially in one instance on an animal which showed a minimal amount of tissue loss from the freezing injury. The low survival rate appears to be due to the fact that the vascular changes following thawing are such that even normal skin cannot be kept alive when applied to this damaged bed.

The survival of grafts on areas B and C are seen to be much lower than on area A although a similar bed was used in each case. The skin applied to areas B and C however was frozen as free skin grafts and this may indicate that these sections received a more severe injury than the skin frozen *in situ* and applied to area A. The latter may have been warmed slightly from the deeper tissues and in this way may not have dropped to as low a temperature as the isolated skin. The skin on areas B and C must obviously have been damaged directly by cold and the different survival rates on B and C must correlate with the post freezing handling of these grafts.

The P^{32} activity in the legs of the dogs in Experiment 2 was taken as an index of the blood flow to the skin of the normal and cold injured areas. These recordings were relative and applied only to individual dogs but by representing the readings in graphic form it was very evident that a variation in discrepancy of blood flow in the control and injured limbs was present. It was interesting to note that in a 14 day follow up the dogs with the greatest tissue loss were those which had shown the greatest discrepancy in blood flow between the limbs in the first days immediately after sustaining the cold injury.

SUMMARY AND CONCLUSIONS

Assessment of survival of frozen skin grafts thawed at room temperature and subsequently transplanted on normal beds suggests that direct injury by cold produced irreversible damage. The survival rate was even lower when rapid thawing was used.

Skin frozen *in situ* however survived well when transplanted to a normal bed while the remaining skin invariably became necrotic. This suggests that the direct injury by cold was not severe enough to destroy the tissue but when the secondary sequelae due to the vascular changes on thawing were added necrosis became inevitable.

Using radio active phosphorus the blood supply to the cold injured part was determined. It was possible to correlate the amount of isotope difference in P^{32} activity in the frozen and control extremities with the subsequent extent of tissue necrosis that occurred.

It is concluded therefore from this investigation that severe cold can produce damage directly when applied to isolated tissue. With skin *in situ*, however cold can produce structural changes but the factor of ischemia during and after thawing is an important etiologic agent in the production of massive tissue necrosis.

Esophagus, Stomach and Small Bowel

UTILIZATION OF THE ILEOCECAL VALVE AS A SUBSTITUTE FOR THE CARDIO ESOPHAGEAL SPHINCTER *

An Experimental Study

JOHN S. NAJARIAN, DWIGHT H. MURRAY, JR., C. D. BUSTER
AND ORVILLE F. GRIMES

Considerable experimental and clinical investigation has been directed toward elimination of the complication of esophagitis that follows excision of the esophagogastric junction. Previous studies have been directed primarily toward either the surgical construction of a valve with autogenous tissue^{1, 2} or the interposition of a segment of jejunum between the esophagus and stomach.³ The latter procedure has protected the esophagus both clinically and experimentally but resulted in some degree of jejunitis.⁴

In order to make a more direct approach to the problem, the following series of experiments was done in which a naturally occurring intestinal valve was substituted for the excised or pathologically destroyed cardiac sphincter mechanism. This experimental study was designed to evaluate the protection afforded the esophagus by interposition of the ileocecal valve and the effect of acid peptic digestion on the interposed colon.

METHOD

Eighteen adult mongrel dogs were used in this study. Each animal was given 1 gm. of neomycin sulfate daily by mouth for 2 days prior to the operation. All operations were performed with general anesthesia produced by intravenous administration of pentobarbital sodium solution and strict aseptic precautions. Excision of the esophagogastric junction and a supra-diaphragmatic vagotomy were performed through a left combined thoraco-abdominal incision. The terminal esophagus was resected superiorly at the level of the left pulmonary vein. The ileocolic segment including 20 to 25 cm. of terminal ileum and approximately 2 to 3 cm. of colon was isolated and the ileocolic vessels were preserved as its blood supply (Fig. 1a). This ileocolic segment was then transposed through the greater omentum to a retrogastric position (Fig. 1b) and anastomosis was accomplished between the terminal ileum and esophagus (Fig. 1c). The colon was anastomosed to the stomach at the site of excision of the cardioesophageal junction (Fig. 1d).

Because of the vagotomy, Finney pyloroplasty was done in 8 dogs (Group 1) and Rammstedt pyloromyotomy in 8 animals (Group 2) in order to obtain adequate gastric emptying. Postoperatively the animals were given 600,000 units of penicillin and one half gm. of streptomycin daily for 4 days. In

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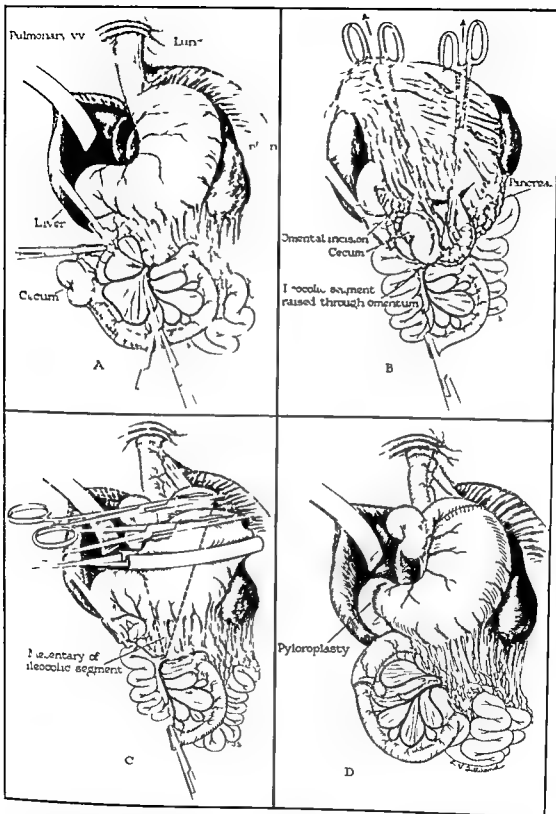


Fig 1 Illustrations of the technique of substitution of the cardioesophageal junction with the ileocecal valve (A) The dotted lines indicate the extent of excision of the terminal esophagus and cardia of the stomach. The ileocolic segment has been isolated with preservation of the ileocolic vessels (B) The greater omentum has been isolated with the ileocolic segment transposed through the lesser sac (C) The esophagoileal anastomosis has been completed and the colon gastric anastomosis is about to be done (D) The colon gastric anastomosis and a Finney pyloroplasty have been completed. Gastrointestinal continuity is established with an end to end ileocolostomy.

travenous fluids were administered for the first 3 days after which fluids were permitted by mouth. By the fifth day all the dogs were taking the regular kennel diet diluted with 1 part milk.

Competency of the ileocecal valve was determined 30 days postoperatively at which time the animal was fed a barium meal through a gastric tube and then anesthetized with pentobarbital sodium. With the dog in the Trendelenberg position the possibility of reflux of barium from the stomach into the esophagus was tested by roentgenologic examination.

After a 6 wk postoperative recovery period all of the animals except 2 were given daily subcutaneous injections of 30 mg of histamine phosphate in oil and beeswax⁴ for 45 days. They were then sacrificed for examination and photography of the gastrointestinal tract. Two of the animals which are being maintained for long term study were not given prolonged histamine stimulation.

The competency of the ileocecal valve was again determined at autopsy by inflation of the excised stomach with water. The transposed valve was considered to be competent if it withstood an intragastric pressure of 50 cm of water.

Histological sections were taken from all the specimens through 5 representative areas. These included (1) the esophagus 4 cm proximal to the esophago ileal anastomosis, (2) the esophago ileal anastomosis, (3) the center of the ileal segment, (4) the ileocecal valve and (5) the cologastric anastomosis. If any other areas of inflammation or ulceration were observed microscopic sections were obtained.

RESULTS

After prolonged stimulation with daily doses of histamine no evidence of either gross or microscopic inflammation was found in the terminal ileum proximal to the ileocecal valve. The short segments of colon distal to the valve were inflamed to varying degrees in 6 out of 16 dogs and were more resistant to acid peptic digestion than either the stomach or the duodenum (Fig. 2).

Inflammation of the transposed segment of colon occurred only one half as frequently in Group 1 (Finney pyloroplasty) as in Group 2 (Rammstedt pyloromyotomy). This may be attributed to the more adequate gastric drainage accomplished with the Finney pyloroplasty. There were only 2 cases of gross ulceration in the transposed segments of colon and in both cases an inadequate stoma between the colon and stomach was found at autopsy. Because of the fact that the ulcers in both instances occurred on the wall of the colon directly opposite the small cologastric stoma it appears possible that the ulcers may have resulted from the action of the jet stream of acid chyme on these particular areas of transposed colon.

Roentgenologic examination *in vivo* or water pressure tests (below 50 cm of water) on the specimens at autopsy indicated that all the ileocecal valves were competent. Pressure exceeding 50 cm of water resulted in incompetence of the valve. This finding was confirmed by the animals' ability to vomit after administration of morphine. (This may be important because while the cardioesophageal sphincter mechanism must prevent reflux it must also be capable of relaxing when vomiting occurs.)

The length of terminal ileum that could be mobilized without difficulty

Fig 2 Photograph of a pathology specimen showing severe gastritis and duodenitis with both gastric and duodenal ulcers after 22 days of stimulation with histamine. No evidence of gross or microscopic inflammation was found proximal to the ileocecal valve.



varied between 15 and 25 cm and the segments could be used for esophageal replacement as high as the mid esophagus. The vascular anatomy of the ileocolic region invariably allowed its mobilization. Near the ileocecal valve the ileocolic artery divided into a colic branch which passed upward on the ascending colon and an ileal branch that ran along the mesenteric border adjacent to the terminal ileum and joined the superior mesenteric artery. Isolation of a long ileal segment together with the cecum and very little accompanying mesentery was thereby accomplished without difficulty.

The 2 dogs being maintained for long term study are now 11 months post operative and show no evidence of esophagitis as determined by esophagoscopy.

SUMMARY

The transposed ileocecal valve was utilized as a replacement for the esophagogastric junction in 18 dogs. The effects of long periods of histamine stimulation on the interposed ileocolic segments were observed. No evidence of inflammation was found proximal to the ileocecal valves and the small colonic segments distal to the valves were more resistant to acid peptic digestion than either the stomach or duodenum.

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THE PREVENTION OF ESOPHAGITIS AFTER ESOPHAGOGASTRIC PYLOROMY IN DOGS BY AN INTERPOSED PLDICAL PYLORUS*

PAUL T. MCGANNON, CARL WILLIAMS, AND STANLEY R. FRIESEN

Reflux esophagitis may occur as a disabling complication following operative procedures in the esophagogastric area for both benign and malignant diseases.¹⁻³ Normally the esophagogastric junction is remarkably competent in protecting the susceptible esophageal mucosa from the injurious action of the gastric chyme.⁴ This protection has been attributed to a number of components of the esophagogastric junction, and include the diaphragmatic pinchcock,⁴ esophagogastric angle, and the gastroesophageal sphincter mechanism.⁵ In the dog, competence of the esophagogastric junction is dependent primarily upon an intrinsic sphincter, independent of normal diaphragmatic attachments.⁷

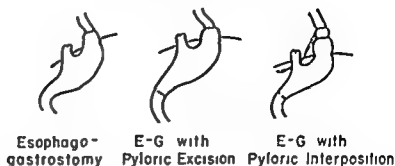
There have been several experimental and clinical operative procedures described for the prevention or treatment of esophagitis. Watkins advocates the reconstruction of the esophagogastric junction, when possible, to restore competence in this area.⁸ Ellis has used the combined procedure of antrectomy, cardiotomy, and vagotomy.⁹ Burford and Lischer have reported a series of patients with esophagitis without stricture treated simply with Linney pyloroplasty.¹⁰ Surgically devised valves have been used to prevent esophagitis.¹¹⁻¹³ An isoperistaltic segment of jejunum to simulate sphincteric action has been substituted for the esophagogastric junction experimentally and clinically.¹² It would be desirable to substitute a true sphincter for the esophagogastric sphincter to protect the esophagus when this junction is diseased or surgically excised. It is the purpose of this presentation to report the results of the interposition of the pylorus between the esophagus and stomach as well as pylorotomy in the prevention of experimentally produced esophagitis in dogs.

The pylorus is suitable as a substitute by virtue of the fact that it is a proven anatomic and physiologic sphincter in close proximity to the esophagogastric area which can be isolated preserving its blood and nerve supply. McCrea has described a special branch of the vagus from the anterior vagal trunk to the pylorus.¹⁴ Wheldon and Thomas have shown that even when denervated the pylorus is capable of rhythmic tonicity.¹⁵ The

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ESOPHAGITIS

Fig. 1 Diagrammatic representation of experimental preparations in Series 1, 2 and 3



pylorus of the dog, isolated with its neurovascular pedicle and re sutured to its normal gastric and duodenal attachments appears to function normally

METHOD

Adult mongrel dogs ranging in weight from 10 kg to 17 kg were used in 3 series of experiments (Fig. 1) *Series 1* Seventeen dogs were subjected to transection of the terminal esophagus 3 cm above the diaphragm with inversion closure of the distal end and anastomosis of the proximal end to the fundus of the stomach *Series 2* In 8 dogs the esophagogastric junction was similarly bypassed by esophagogastrostomy and in addition pyloric excision with gastroduodenostomy was carried out *Series 3* In 13 dogs the distal esophagus was similarly divided the distal end closed and the isolated pylorus with its neurovascular pedicle was interposed between the proximal esophagus and the fundus of the stomach Distal continuity was established by gastroduodenostomy

All operations were performed with sterile technique using intravenous sodium pentothal anesthesia (15 mg/lb of body weight) Respiration was controlled with endotracheal oxygen and an automatic respirator The left transthoracic incision through the ninth interspace was used in each instance Anastomoses were in 2 layers with a running 3/0 plain catgut mucosal suture and interrupted 1/0 silk seromuscular sutures The diaphragm was incised anteriorly apart from the esophageal hiatus to permit adequate exposure of the pylorus The pylorus with a small margin of antrum was isolated together with the right gastric artery or its branches in the gastrohepatic ligament and rotated 90° laterally when interposition was carried out Care was taken not to constrict the blood supply to the pedicled pylorus in the closure of the diaphragm about the gastric fundus The vagus trunks were left intact in all series of dogs

All animals in the 3 series were given only water for 2 days followed by a liquid food preparation and started on solid food at 1 wk postoperatively All dogs were weighed at regular intervals Esophagoscopy under general anesthesia was done at intervals in many of the dogs Antibiotics were given for one week At death or sacrifice of the animal its gross and microscopic observations of the esophagus, stomach and duodenum were recorded

RESULTS

Series 1 (See Table 1) Sixteen of 17 dogs which had bypass esophagogastrostomy without pyloric excision or interposition had developed esophagi-

Table 1 Series 1 Dogs with By pass Esophagogastrostomy Only

DOG NO	DAYS	FINDINGS	REMARKS
119	14	No esophagitis	Died
120	20	Severe esophagitis with perforation	Died
119	28	Esophageal erosion	Died
115	29	Moderate esophagitis	Died
103	30	Esophagitis	Died
76	39	Severe ulcerative esophagitis	Died
70	40	Esophagitis	Died
133	56	Esophagitis	Died
43	64	Mild esophagitis	Sacrificed
47	65	Moderate esophagitis	Sacrificed
52	68	Esophagitis	Died
124	71	Moderate esophagitis	Sacrificed
114	76	Moderate esophagitis	Died
118	78	Moderate esophagitis	Sacrificed
113	78	Moderate esophagitis	Sacrificed
14	79	Severe esophagitis	Sacrificed
117	82	Moderate esophagitis	Sacrificed

Table 2 Series 2 Dogs with By pass Esophagogastrostomy and Pylorectomy

DOG NO	DAYS	ESOPHAGOSCOPY	AUTOPSY	REMARKS
16	25	Esophagitis	No esophagitis	Died
73	30	Esophagitis	Mild esophagitis	Sacrificed
122	40	Mild esophagitis	No esophagitis	Died
128	42	Esophagitis	No esophagitis	Sacrificed
14	60	Esophagitis	No esophagitis	Died
124	68	Mild esophagitis	Moderate esophagitis	Sacrificed
126	69	Esophagitis	No esophagitis	Sacrificed
81	81	Esophagitis	No esophagitis	Sacrificed

was when sacrificed 11 to 82 days after operation. The one dog which did not develop esophagitis died 14 days after operation because of thoracic empyema due to operative contamination.

Series 2 (See Table 2) Eight dogs with by pass esophagogastrostomy and pylorectomy had evidence of esophagitis on repeated esophagoscopy examinations at some time prior to sacrifice while only 2 of the 8 had esophagitis as a gross finding at the time of autopsy. The transitory esophagitis was mild to moderate in degree and was noted by several observers. The average weight loss in this series of dogs was 34 per cent.

Series 3 (See Table 3) None of the 13 animals with the pedicled pylorus interposed between the esophagus and stomach had evidence of esophagitis 14 to 91 days following the procedure. No esophagitis was seen on esophagoscopy even though this procedure was done repeatedly in 4 of the dogs. One animal died 29 days postoperatively from a perforated duodenal ulcer.

Table 3 Series 3 Dogs with Interposition of the Pedicled Pylorus Between the Esophagus and Stomach By pass

DOG NO	DAYS	FINDINGS		
		ISOIHAGOSCOPY	AT TORSY	REMARKS
129	14	Not done	No esophagitis	Died
11	18	Not done	No esophagitis	Sacrificed
18	19	Not done	No esophagitis	Sacrificed
76	20	Not done	No esophagitis	Sacrificed
98	20	Not done	No esophagitis	Died
15	26	Not done	No esophagitis	Sacrificed
71	29	Not done	No esophagitis	Died perforated duodenal ulcer
57	31	No esophagitis	No esophagitis	Sacrificed
41	46	No esophagitis	No esophagitis	Sacrificed
105	83	No esophagitis	No esophagitis	Sacrificed
99	84	No esophagitis	No esophagitis	Sacrificed
132	87	No esophagitis	No esophagitis	Sacrificed
150	91	No esophagitis	No esophagitis	Sacrificed

no esophagitis was found. The average weight loss in this series was 18 per cent.

A significant number of dogs died in the immediate and early postoperative period (not listed in Table) because of gangrene of the pylorus due to technical errors in maintaining a good blood supply through the vascular pedicle. Constriction of the pedicle at the level of the diaphragm can be avoided by meticulous attention to closure of the diaphragm about this pedicle.

DISCUSSION

When the pylorus was placed between the esophagus and the stomach by passing the esophagogastric splincter there was no evidence whatsoever of esophagitis. The results of this study suggest that this protection may be due in part to absence of pyloric resistance at the distal end of the stomach and in part to its interposition separating the stomach from the esophagus. Although pylorectomy without interposition demonstrates a significant decrease in the severity and incidence of esophagitis, the addition of pyloric interposition yields a uniform protection. The weight loss in the pylorectomy group of dogs was noticeably greater than in the series when pyloric interposition was added. The reason for this difference is not known. The mortality rate on the other hand is greater in the group in which the pylorus is pedicled and interposed. The significant though not complete protection attending pylorectomy in this study supports the clinical experience of Lischer and Burford that Finney pyloroplasty benefits patients with esophagitis.

Just prior to the time of sacrifice of the dogs with the interposed pedicled pylorus a number of these animals were anesthetized so that observations of the pylorus in this location could be made. Definite contractions of the pylorus could be seen which also responded to direct electrical stimulation but not to vagal trunk stimulation. Detailed studies of pyloric function are being done. Minimal organic obstruction at the interposed pylorus was noted in only one dog.

It is interesting that 1 dog (No 71) with pyloric interposition died 29 days following operation because of a perforated duodenal ulcer. This dog had appeared to be in good health and had lost only 2 per cent of its body weight. No other acid peptic changes were noted in the stomach or duodenum of any of the other dogs. It would be interesting to know if chronic histamine or bethane stimulation would increase the incidence of esophagitis in the experimentally protected animals.

CONCLUSIONS

- 1 Esophagitis is a common finding in dogs following esophagogastrostomy which bypasses the esophageal gastric junction.
- 2 When a pylorotomy is combined with the esophagogastrostomy esophagitis of a transitory nature is seen to develop which subsides in time.
- 3 No evidence of esophagitis was noted in the dogs with a pylorus interposed between the esophagus and gastric bypass.

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Addendum. At the time of the presentation of this paper 13 dogs were included in Series III of the experiments 4 dogs of which demonstrated esophagitis at the time of autopsy.

THE SIGNIFICANCE OF INNERVATION IN THE FUNCTION OF THE GASTRIC ANTRUM*

H. A. OBERHEIMAN, JR., STANLEY P. RICLER AND LESTER R. DRACHSTEIN

It has been established both experimentally and clinically that the gastric mechanism remains active after exclusion of the gastric antrum from the remainder of the stomach providing that distension of the antrum by regurgitated food and intestinal content occurs.¹ However, total isolation of the denervated antrum abolishes the gastric phase of secretion.² It seemed of interest to study the behavior of the antrum with intact innervation after total exclusion from the gastrointestinal tract.

METHOD

Heidenhain pouches were constructed in 1 dog. After recovery from the operative procedure control studies were obtained. The antrum was then excluded from the body of the stomach by a mucosal bridge leaving the entire seromuscular liver, lesser curvature structures and nerve supply intact. The pylorus was divided and a cutaneous fistula constructed from the proximal end. Gastrointestinal continuity was reestablished by gastroduodenostomy (Fig. 1).

Following recovery from the operation quantitative daily collections of gastric juice were resumed using the methods previously described from this laboratory. The mean daily output of hydrochloric acid during a 30 day collection period was expressed in mEq. The secretory response to feeding a standard meal fasting for a 3 day period and the intravenous administra-

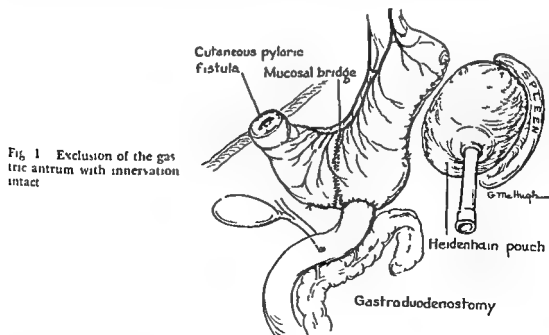


Fig. 1 Exclusion of the gastric antrum with innervation intact

*From the Department of Surgery, the University of Chicago School of Medicine. Aided by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and from the Otho S. A. Sprague Memorial Institute.

tion of 15 units of regular insulin were measured on multiple occasions. Antrum motility was studied by introducing a small Foley balloon connected to a strain gauge and recording system through the cutaneous fistula. Observations were made under conditions of feeding, fasting, and after the administration of insulin.

RESULTS

Following total exclusion of the innervated antrum, the quantitative 24-hour output of hydrochloric acid remained essentially unchanged in all 4 animals. The response to insulin hypoglycemia was negative in all 4 dogs during the control period. However, after exclusion, repeatedly positive tests were seen in 2 animals, and occasional positive tests were obtained in the other 2. When the dogs were fasted for a 3-day period, almost complete cessation of secretion from the Heidenhain pouch was observed, of the same order of magnitude as that which follows intrectomy (Table 1).

Table 1. The Effect of Exclusion of the Innervated Antrum on Gastric Secretion

DOG NO	MEAN 24 HR SECRETION (mEq of HCl)		SECRETORY RESPONSE TO INSULIN HYPOLYCEMIA		MEAN 24 HR SECRETION (mEq of HCl) EFFECT OF FASTING	
	Control	Antrum Excluded	Control	Antrum Excluded	3 Days Control	3 Days Fasting
D 800	34	27	— —	++++		
D 983	23	22	— —	++++	25	1.2
D 994	35	38	— —	+—+—	38	0.7
D 992	25	21	— —	+—	25	1.9

Feeding a standard meal consisting of 200 gm of horsemeat or of a proprietary dog food evoked a marked secretory response which began 15 to 30 min after feeding and lasted for from 4 to 6 hr. The magnitude of the response was as great as that customarily observed in a Heidenhain pouch dog with intact antrum. However, the duration of the response was 2 to 3 times longer than that usually seen.

Motility studies performed during periods of fasting revealed the presence of waves of low amplitude occurring at a rate of 2 to 3/min. Fifteen to 30 min after feeding, rhythmical contractions of the antrum of much greater amplitude and increased frequency commenced and persisted during the entire secretory phase. Occasionally a brief spurt of activity was observed immediately after the dog had eaten, followed by the usual motility pattern. These were felt to represent vagally induced contractions.

Motility studies were also performed following the administration of insulin. Antral contractions were usually observed from 30 to 60 min after the drug had been given, at the time that the hypoglycemic reaction was most marked (Fig 2).

DISCUSSION

If its innervation be preserved, the gastric antrum continues to function despite complete exclusion from gastrointestinal continuity. Indeed, the antral phase of secretion, as measured by quantitative daily secretion of gastric

ANTRUM MOTILITY IN RESPONSE TO FEEDING AND INSULIN HYPOGLYCEMIA

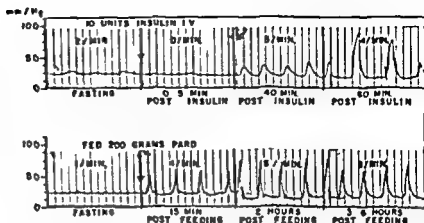


Fig. 2 The effect of feeding and insulin hypoglycemia on antrum motility

juice from denervated pouches remains as great as before exclusion. The nature of the preparation eliminates the possibility that regurgitated food or intestinal content is responsible for this phenomenon. What then is the mechanism?

Previous investigations have suggested that distension and peristalsis of the antrum initiate the release of gastrin.³ In the studies reported here it would appear that those events which induce motility of the antrum lead to production of gastrin as demonstrated by secretion of gastric juice from the antrum phase pouch. Thus feeding the animals which would be expected to induce vagal stimulation of antrum motility as well as peristalsis transmitted by way of the intact intramural autonomic reflexes evokes a secretory response while fasting with its concomitant decrease in peristaltic activity of the stomach results in a virtual cessation of antral phase secretion. Insulin hypoglycemia leading to parasympathetic stimulation produces a definite although small secretory response. Motility studies confirm that those phenomena which induce secretion of gastrin origin are associated with antrum motility.

It is of interest that the quantitative daily secretion of these animals is little affected by total exclusion. One might infer that the actual presence of food in the antrum contributes nothing to the elicitation of antrum function. This inference is probably not justified since the pH of the antrums under investigation here is neutral and the inhibitory effect of acid which obtains in the normal dog is not active in these animals.

SUMMARY AND CONCLUSIONS

Total exclusion of the gastric antrum from intestinal continuity with preservation of its intrinsic and extrinsic nerve supply does not abolish or quantitatively decrease the gastric phase of secretion. Those phenomena which lead to motility of the antrum are associated with a secretory response of antrum origin. It is concluded that motility of the antrum produced by peristalsis is an adequate stimulus for antral phase secretion.

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DOG NO	MEAN 24 HR SECRETION (ml of HCl)		SECRETORY RESPONSE TO INSULIN HYPOGLYCEMIA		MEAN 24 HR SECRETION (ml of HCl) EFFECT OF FASTING	
	Control	Antrum Excluded	Control	Antrum Excluded	3 Days Control	3 Days Fasting
D 800	31	27	— —	++++		
D 983	23	22	— —	++++	25	12
D 984	35	38	— —	+ + + —	38	07
D 992	25	21	— —	+ —	25	19

Feeding a standard meal consisting of 200 gm of horsemeat or of a proprietary dog food evoked a marked secretory response which began 15 to 30 min after feeding and lasted for from 1 to 6 hr. The magnitude of the response was as great as that customarily observed in a Heidenhain pouch dog with intact antrum. However the duration of the response was 2 to 3 times longer than that usually seen.

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At a third procedure in this animal the antrum muscularis colon mucosa hybrid antrum was excised and Heidenhain pouch secretions collected.

The remaining 5 animals in the study were reoperated following a suitable control period of collections from the Heidenhain pouches. All muscularis was stripped and excised from the antrum of the stomach utilizing again the submucosal cleavage plane. Muscularis was stripped from the mid colon in two 8 cm lengths with individual blood supply. The stripped colon mucosa was excised and continuity re-established by end to end anastomosis. The 2 colon muscularis strips were then sutured to the bare antrum mucosa as an anterior and a posterior patch completely covering it in an antiperistaltic fashion. At all times the bare mucosa and muscular strips appeared pink and viable. Secretions from the Heidenhain pouches were again collected for a suitable period.

At a third procedure in these 5 animals the hybrid antrum was excised from the stomach and transplanted to the midcolon as a diverticulum. Gastroduodenostomy re-established gastrointestinal continuity. This simulates with the antrum mucosa colon muscle hybrid antrum the antrum to colon preparation. Twenty four hour quantitative secretions were again collected, volume and free acid determined. Two of the animals died following this procedure.

In 2 of the 5 animals a fourth procedure was completed. The hybrid antrum diverticulum of the colon was resected from the colon fitted with a cannula and closed. The antrum cannula was brought out through a right abdominal stab wound and the colon defect was sutured closed. The animal in this manner was converted to a Heidenhain pouch hybrid antrum pouch preparation. It must be noted that the blood supply of this hybrid antrum during the last 2 procedures was derived entirely from the vessels supplying the transplanted colon musculature and these vessels were carefully preserved throughout.

All of the animals remained healthy throughout the study periods indicated except as stated. Diets were changed at no time except for the immediate postoperative periods varying from 3 to 7 days. During the immediate postoperative period no pouch collections were made. Penicillin was administered routinely for the first 3 days postoperatively.

RESULTS

It is apparent in D 52 that the antrum musculature is unable to release gastrin. If it were the site of gastrin release its position covering a diverticulum of colon mucosa should cause a hypersecretion from the Heidenhain pouch if we can reason from the antrum to colon preparation. Yet it produced no hypersecretion in fact secretion fell as though the animal had been deprived of its antrum.

Because the musculature of the antrum covering a diverticulum of colon mucosa and thus in a position of maximal stimulation produced no response from the Heidenhain pouch the reverse situation was prepared. Antrum mucosa was stripped of its musculature and covered with colon muscle placed antiperistaltically in an attempt to produce gastric stasis and give this preparation every opportunity to demonstrate an antrum phase of secretion. Surprisingly secretions from the Heidenhain pouches in these animals also dropped to levels comparable to that produced by antrectomy. It should

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STUDIES ON THE SITE AND MECHANISM OF GASTRIN RELEASE*

C M BAUGH J BARGENA J BRAVO AND L R DRAGSTEDT

The importance of the gastric antrum as the site of release of a humoral stimulant of gastric secretion has been demonstrated and elaborated upon in a variety of ways. Distention of the antrum¹ and acetylcholine² or liver extract applied locally to the antrum are known to release gastrin. Locally applied acid, topical anesthetics and systemic atropine³ are known to inhibit the gastrin mechanism. Transplantation of the antrum to the colon as a diverticulum⁴ is a powerful stimulant of gastric secretion via the gastrin mechanism. However the mechanism of release of this humoral agent called gastrin and its site of release in the antrum have not been demonstrated.

In order to study this problem it was decided to isolate antrum muscle from mucosa and follow quantitative 24 hr secretions from a denervated pouch (Heidenhain pouch).

METHOD

In each of 6 healthy adult mongrel dogs a Heidenhain pouch was constructed in the usual manner and fitted with a stainless steel cannula which was brought out through a left abdominal stab wound. The cannulae were fitted with football bladders and 24 hr secretions were collected quantitatively. Free hydrochloric acid was determined by titration with one tenth normal sodium hydroxide using p dimethylaminoazobenzene as indicator.

After a suitable control period of collections from the Heidenhain pouch one of the animals was reoperated. The stomach was transected at the antrofundic junction and all antrum mucosa was stripped out utilizing the submucosal cleavage plane. The antrum mucosa was excised in the pylorus and the open mucosa of the duodenum infolded by suture. The bare antrum muscle receiving its blood supply from its attachment to the duodenum was then transplanted to cover a surgical diverticulum of the colon mucosa to form an antrum muscle-colon mucosa hybrid variation of the reported antrum to colon propritation. Gastrojejunostomy reestablished gastrointestinal continuity. Twenty four hour collections from the Heidenhain pouch were again followed.

*From the Department of Surgery of The University of Chicago. Aided by grants from the Otho S. A. Sprague Memorial Institute and from the Division of Research Grants and Fellowships of the National Institutes of Health U.S. Public Health Service.

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Table 1 Average daily volume clinical units and mEq of free acid secreted from the Heidenhain pouch during a control period hybrid antrum period after the hybrid antrum had been transplanted to the colon, and after the animal had the antrum excised or converted into a pouch Note that D 52 is a different preparation than the others listed

DOG NO	HEIDENHAIN POUCH CONTROL PERIOD				HYBRID ANTRUM				HYBRID ANTRUM TRANSPLANTED TO COLON				ANTRECTOMY OR ANTRUM POUCH			
	AVR VOL	FREE ACID	mEq	# OF COLL	AVR VOL (cc)	FREE ACID	mEq	# OF COLL	AVR VOL (cc)	FREE ACID	mEq	# OF COLL	FREE ACID	mEq	# OF COLL	
D 168	196	81	110	90	64	72	46	34	132	105	137	37	87	92	31	
D 172	416	117	497	30	68	81	30	46	(Dead)	
D 202	672	133	894	44	134	93	143	30	473	119	563	20	(Dead)	
D 206	161	117	188	47	74	61	45	32	199	110	219	35	43	22	31	
D 280	239	118	282	27	94	71	67	31								
D 52	197	110	437	39					72	154	11	44	80	72	58 51	

be noted that the pouch secretions showed no tendency to rise toward the end of the collection periods in any of these animals. Temporary vascular embarrassment was thus excluded as an explanation for the drop in secretions.

With no activity of the gastrin mechanism evident from the antrum mucosa while remaining in its normal relation to the stomach, it appeared that neither the antrum musculature nor the antrum mucosa was separately capable of releasing gastrin. However, doubt remained that both layers should be inactive. Therefore, it was decided to transplant this antrum mucosa-colon muscle hybrid antrum to the colon as a diverticulum in order that the site of gastrin release be in a position of maximal stimulation.

Following transplantation of the antrum mucosa-colon muscle hybrid antrum to the colon, an increasing increase in 24 secretions from the Heidenhain pouches occurred, indicating that the gastrin mechanism was present and active at least in this location. However, it must be emphasized that these animals did not hypersecrete with the hybrid antrum in this position. This together with the fact that there was little or no evidence of antrum function with the hybrid antrum in its normal position in the stomach suggests that the surgery traumatized the antrum to such an extent that greater stimuli were required to activate the gastrin mechanism of the hybrid antrum than are normally present in a dog's stomach.

A final confirmatory procedure of constructing a pouch of the hybrid antrum diverticulum was done after resecting it from the colon. In the animals surviving this procedure, secretions from the Heidenhain pouches again dropped much as one would expect if working with a normal antrum. In this position the normal antrum is effectively nonfunctioning and this proved to be true of the antrum mucosa-colon muscle hybrid antrum.

DISCUSSION

Four items were established at this point: (a) gastrin is released from one or a combination of structures in the mucosa and submucosa; (b) gastrin can be released in the absence of the antrum musculature and its contained nervous structures; (c) the gastrin mechanism remains quite viable and is responsive despite these extensive surgical procedures; (d) in order to maintain a normal gastrin mechanism, the antrum layers must remain intact.

With the gastrin mechanism thus localized to the antrum mucosa and submucosa, speculation as to the cellular site of gastrin can reasonably be limited to 2 structures: e.g. mucosal cells and/or nervous tissue such as Meissner's plexus. The lack of histological differentiation of the antrum mucosa cells by present techniques allows suspicion to point more strongly toward nervous tissue as the site of gastrin release. This is supported by the manner in which the antrum responds to acetylcholine, topical anesthetics, and systemic atropine. The release of gastrin from nervous structures could either be direct or mediated by a local reflex arc.

SUMMARY

The procedure of making a hybrid gastric antrum, either of antrum muscle and colon mucosa or antrum mucosa and colon muscle, has been described. Hybrid antrums prepared in this manner demonstrated that the antrum muscularis, containing its nervous plexus, is not the site of formation

or release of gastrin. Gastrin is released from the gastric antrum mucosa and/or submucosa. The suggestion is presented that gastrin is released from Meissner's plexus or other nervous structures in the antrum submucosa or mucosa either by direct stimulation or by way of a local reflex arc.

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INHIBITION OF THE INTESTINAL PHASE OF GASTRIC SECRETION BY ANTRAL INHIBITOR SUBSTANCE*

BENJAMIN D. MARGOLUS AND R. CAMERON HARRISON

Dragstedt and his associates demonstrated the influence of pH in the region of the antrum on gastric secretion. Having shown that the presence of acid in the antrum depressed acid production by the body of the stomach, they concluded that this action was entirely due to a reduction in gastrin production by the antrum. Other workers have suggested that the antrum might secrete an inhibitor substance. Previous experimental work done in our laboratory demonstrated this and further indicated that the mode of action of this substance is humoral. However its site of action has not been established and its possible influence on other phases of gastric secretion has not been clarified. The following experiment suggests that this antral inhibitor is also effective against the intestinal phase of gastric secretion.

METHOD

Modifying a preparation described by Woodward and his associates,³ dogs were prepared with Heidenhain and total antrum pouches. The whole antrum was separated from the body of the stomach, excising the junctional area to ensure that no acid-secreting mucosa remained in the antral pouch. A stainless steel cannula was placed in the antrum and the pyloric end of the antral pouch was brought through the abdominal wall as a mucocutaneous fistula. The duodenal stump was closed and gastrointestinal continuity reestablished by anastomosing the main stomach to the distal portion of the duodenum (Fig. 1).

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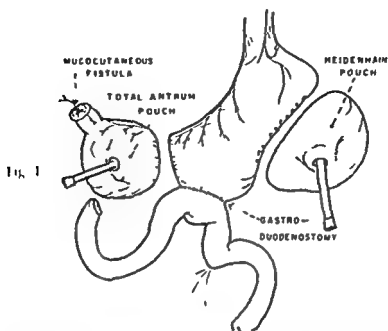


Fig. 1

After a recovery period of 3 to 4 wks during which time the dogs were trained to stand in a Pavlov frame the antral pouch secretion was tested for acid following the injection of a stimulating dose of histamine (1 mg). Invariably the secretion that followed contained no free HCl so we were confident that no acid producing mucosa had been left in the antrum.

The dog in the fasting state was placed in the Pavlov frame and the antral pouch continuously irrigated with N/10 HCl. After 30 min the animal was fed a standard meal consisting of 1 can of dog food and Heidenhain pouch secretion collected for 4 hr. During the control experiment the ani-

Table 1

		ANTRUM IRRIGATED WITH 0.9% NaCl		ANTRUM IRRIGATED WITH N/10 HCl	
		VOLUME cc	HCl mEq/l	VOLUME cc	HCl mEq/l
Dog A	Test No 1	39	194	15	8
	2	23	192	10	4
	3	26	119	20	4
	4	21	190	05	0
	5	58	222	05	0
Dog B	Test No 1	60	153	30	4
	2	20	198	25	0
	3	35	156	40	14
	4	10	72	10	0
Dog C	Test No 1	34	138	40	0
	2	19	102	20	0
	3	22	156	70	6
	4	17	81	15	4
	5	10	100	50	0

mal again in the fasting state was placed in the Pavlov frame and the routine of feeding and collection of Heidenhain pouch secretion was repeated. However on this day the irrigating solution used was normal saline. Several determinations with both irrigating solutions were made in each of the 3 dogs.

RESULTS

Perfusion of the antral pouch with N/10 HCl resulted in marked diminution of Heidenhain pouch secretion. The amount of acid in the secretion was markedly reduced or totally inhibited in all determinations (Table 1).

DISCUSSION

In this preparation the Heidenhain pouch was denervated and the antrum completely separated from the remainder of the gastrointestinal tract. The secretory response of the Heidenhain pouch following the ingestion of food could therefore be presumed to represent intestinal phase secretion.

Since it had previously been shown that mechanical distention of the antrum stimulates gastric secretion during our control determinations normal saline was used to perfuse the antral pouch. However the perfusing solutions did not appear to stimulate secretion mechanically as Heidenhain pouch secretion was not found to vary significantly with or without saline perfusion.

This experiment would indicate that the antrum in an acid environment produces an inhibitor substance capable of inhibiting the intestinal phase of gastric secretion.

THE EFFECT OF CORTISONE ON HISTAMINE STIMULATION OF GASTRIC SECRETION IN THE ADRENALECTOMIZED DOG*

BERNARD SIGEL, JAMES G. BASSETT AND DONALD R. COOPER

With the addition of ACTH and cortisone to medical therapeutics there have been many reports of exacerbations of symptoms and complications of peptic ulcer in individuals receiving these steroids. The work of Gray^{1, 2} indicates that an increase in gastric secretion which he has observed following administration of these hormones may be responsible for this occurrence. The means by which this increase of gastric secretion is effected, however, is not clear. Whether cortisone exerts a direct influence on parietal cells, plays a permissive role in relation to other mechanisms, or exerts an indi-

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With the technical assistance of Janet A. Parker and Elsa D. Kertesz.

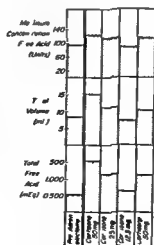
rect effect involving its action on water and electrolyte metabolism seem to be important possibilities which warrant consideration. Thus in a preliminary report of a study which proposes to elucidate certain actions of cortisone on gastric acid secretion in adrenalectomized dogs. In this way, the specificity of cortisone can be observed particularly in comparison to other forms of adrenal cortical replacement and also during controlled variation of other conditions.

METHOD

Mongrel dogs weighing 20 to 25 lbs were used. Small Pyloric pouches were constructed in 4 animals and insulin testing revealed 1 of these to be innervated and 3 deinnervated. Histamine pouch analyses were then performed in the following manner: 1) the animals were fasted for at least 18 hr and the tests were performed in the morning; 2) the basal pouch secretions were tested and in nearly all instances were found to contain no free acid; 3) histamine diphosphate 0.5 mg was injected subcutaneously; 4) the animals were trained to stand or sit quietly while pouch collections were obtained every 15 min for 90 min; 5) the collected specimens were titrated for free and total acid. The dogs were fed a standard laboratory diet following the tests and unless certain conditions of the experiment called for it this was not supplemented or substituted. Following these base line studies single stage bilateral total adrenalectomies were performed utilizing lumbar approaches. One week after adrenalectomy the animals were again subjected to histamine pouch analyses similar to those conducted during the base line period. These tests were now performed under varying conditions of adrenal cortical replacement. At least 18 hr were allowed for each dog to be acclimatized to a new condition before testing was begun and 2 to 8 histamine tests were performed during each period. The only exception to this was when adrenal crisis was induced in less than 48 hr and histamine testing was performed earlier. Serum electrolyte and urea nitrogen determinations were performed during most of the test conditions.

RESULTS

The total mEq of free acid secreted was markedly increased when the adrenalectomized animals were receiving 50 mg of cortisone. As the dosage



Relationship of Cortisone Dosage to Histamine Induced Gastric Acid Secretion

Fig 1 The effect of cortisone dosage on histamine induced gastric acid secretion

Adrenalectomy in two Heston Pouch Dogs (18 and 108). Average

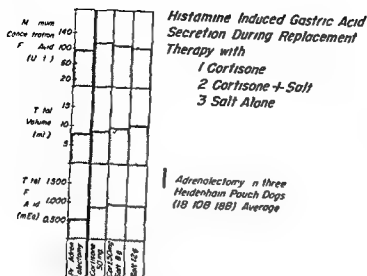


Fig 2 Comparison of cortisone to other forms of replacement therapy

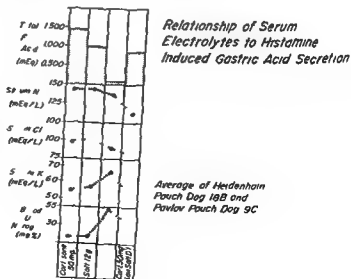
was reduced the free acid produced during the testing period concomitantly decreased. This was mainly a function of volume of gastric juice for the peak concentrations of free acid were only slightly affected. When the cortisone was again increased there was a rise in acid secreted (Fig 1).

When cortisone 50 mg daily was administered the response to histamine was greater than before adrenalectomy. Cortisone 50 mg plus 8 gm of supplementary salt had a comparable effect. The results, when 12 gm of salt only were administered were essentially the same as when cortisone or cortisone plus salt was given (Fig 2).

When no substitution therapy was administered the animals developed more or less vomiting, marked weakness, weight loss, and bloody diarrhea. Gastric secretion at this time was markedly suppressed, the total mEq volume and concentration being equally affected (Fig 3).

When adrenal insufficiency was treated with 50 mg of cortisone daily but the animals maintained on a low salt diet, clinical symptoms ameliorated but serum sodium and chloride remained low. Histamine was capable of evoking a good acid secreting response at this time (Fig 3).

Fig 3 The effect of adrenal insufficiency on gastric acid secretion and the effect of low serum concentrations of sodium and chloride during cortisone replacement therapy



DISCUSSION

Animals submitted to total adrenalectomy provide in excellent preparation for observing certain effects of the adrenal glands on gastric acid secretion. When an adrenalectomized animal is adequately maintained on cortisone or sodium chloride, a powerful stimulant such as histamine can evoke a good secretory response. In fact, when large amounts of cortisone or salt are given, this response is greater than in the intact animal. The reason for this is not made clear by this study. However, it is evident that histamine can produce its effect on gastric acid secretion in the complete absence of cortisone or for that matter the adrenal cortex itself, provided the animal is kept out of acute adrenal cortical insufficiency by salt replacement. In this respect then, cortisone does not play a permissive role. Since only histamine has been tested, it remains for further investigation to determine if this is true for other mechanisms of gastric acid secretion. Such studies are in progress.

Serum concentrations of sodium and chloride do not seem to be the *sine qua non* for acid production by histamine, for this can occur with low concentrations of these electrolytes if cortisone is administered. It appears therefore that histamine stimulated acid secretion in the adrenalectomized dog can occur provided that the deleterious effects of adrenal cortical insufficiency can be circumvented by replacement therapy.

CONCLUSIONS

1. Following total ablation of the adrenal glands the gastric acid secreted in response to histamine in the pouch dog varies directly with the dose of cortisone administered.

2. Dogs exhibiting signs of frank adrenal cortical insufficiency demonstrate marked reduction in both volume and concentration of gastric acid in response to histamine.

3. In the dog the adrenal glands or their products are not a prerequisite for gastric acid secretion induced by histamine.

4. It is unlikely that the effect of adrenal steroids on histamine stimulated secretion of gastric acid is secondary to their action on serum electrolyte concentrations.

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PEPSIN IN GASTRIC PHYSIOLOGY EFFECT OF ULCER DIATHESIS ACTH HISTAMINE, ANESTHESIA OPERATION BANTHINE PILOCARPIN EPINEPHRINE AND SEDATION UPON BLOOD AND URINE PEPSIN LEVELS AND UPON GASTRIC ACIDITY*

ALBERT L. MEENA J HAROLD CONN HANS NAUMANN AND
JAMES D HARDY

During the past 5 to 10 years there has appeared a considerable volume of literature concerning 2 proteolytic enzymes related to gastric pepsin namely uropepsin in the urine and pepsinogen in the plasma Pepsinogen is secreted almost entirely by the chief cells of the gastric mucosa² and approximately 1 per cent gains access to the blood stream and is excreted by the kidneys as uropepsin²

The inter relationship of uropepsin plasma pepsinogen and gastric pepsin has been established by the disappearance of the enzyme from the urine after gastrectomy¹ the decreased concentration in the urine of patients with pernicious anemia^{1 2} the increased activity in the urine of patients with duodenal ulcer^{1 4} and the increased concentration in the urine of dogs after intravenous administration of pepsinogen¹

The work of Mirsky and his associates indicated that the individual values of plasma pepsinogen were fairly constant from day to day varied little at different times of the day were not affected by the ingestion of food were increased in hypersecretory states such as peptic ulcer and were decreased in hyposecretory states such as gastric carcinoma Other investigators have repeated and expanded upon this earlier work

A major aim in the present study was to determine if there were a significant correlation between gastric acidity and the production of pepsinogen and to measure uropepsin and plasma pepsinogen in normal adult male hospital patients as opposed to duodenal ulcer patients It was also planned to determine the effect if any of various drugs and surgical procedures on the production of pepsinogen

METHOD

A modification of the method of Anson⁶ and Mirsky¹ was used to measure plasma pepsinogen in a blood sample and uropepsin in a 24 hr urine specimen The activity of the enzymes was measured by the amount of tyrosine and amino acid liberated by the protein breakdown of an hemoglobin substrate on incubation and determined colorimetrically by a phenol reaction Results are expressed in units^{1 6} Two hundred and fifty six determinations were made in 110 patients

RESULTS

The mean 24 hr uropepsin excretion in 39 normal male adult hospital patients who had no complaints referable to the gastrointestinal tract was

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BLOOD PEPSINOGEN LEVELS IN A NORMAL PERSON FOR 7 CONSECUTIVE DAYS

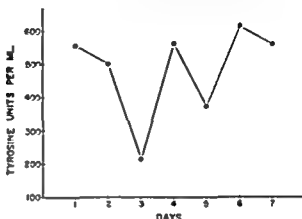


Fig 1 Note the considerable variation in levels of the same individual taken at the same hour each day

4822 \pm 2416 units the mean plasma pepsinogen level was 415 \pm 139 units. In the present study these figures were considered to represent normal values due to modifications of technique they differ somewhat from those reported by others. For instance, Grey *et al* report a mean of 2300 \pm 700 units of uropepsin and Mirsky *et al* report a mean of 600 units of plasma pepsinogen.⁸ However, all groups agree that there is a wide scattering of normal values for both uropepsin and pepsinogen.

In order to detect the daily variations of plasma pepsinogen blood samples were drawn at the same time of day for 7 consecutive days from one of the authors of this paper (ALM). As shown in Figure 1 there is considerable variance of the plasma pepsinogen level from day to day ranging from 215 units to 620 units. These results are in disagreement with Mirsky and others who found a fairly constant day to day level of pepsinogen.

To determine if there were a correlation between gastric acidity and the production of pepsinogen simultaneous measurements of basal gastric acidity, plasma pepsinogen and 24 hr excretion of uropepsin were made on 22 adult male hospital patients. Continuous gastric suction was maintained for 24 hr by a Levin tube and at the same time a 24 hr urine collection was made. During this 24 hr period a sample of blood was drawn for the measurement of plasma pepsinogen. The volume of gastric output and free and total acidity were measured. No drugs were used for stimulation of gastric secretion. In a statistical analysis of the data no correlation was found to exist between the amount of total or free acid and the production of pepsinogen. In other words patients with a high level of gastric acidity did not necessarily have a high production of pepsinogen.

Twenty-one patients who either had proven duodenal ulcers or typical ulcer symptoms were studied. This group as a whole had a higher mean in both uropepsin and blood pepsinogen values than did the normals but there was a great deal of overlapping.

The breakdown in the ulcer group was as follows: in 5 patients with an active duodenal ulcer as proven by clinical and radiologic evidence the mean for uropepsin was about the same as for the normal group and the mean for plasma pepsinogen was slightly higher. In patients who had inactive ulcers both enzyme levels were less than in the normal group. In duodenal ulcer with obstruction both values were increased. In the group that

on the basis of Mirsky's assumption that stress stimulates pepsinogen production.²

Effect of Cortisone, Ephedrine, Pilocarpine, Thorazine, and Probanthine
Twenty-one patients were studied to determine if certain drugs would affect the output of pepsinogen and uropepsin. Control enzyme levels were determined, the drug administered for 5 days, and the levels again determined. In general, both the uropepsin and pepsinogen levels changed proportionately. Cortisone (7 patients) and ephedrine (6 patients) caused a mean decrease in the enzymes, while pilocarpine (5 patients) and thorazine (2 patients) caused an increase. The results with probanthine (1 patient) were equivocal. Where variations occurred, the uropepsin and pepsinogen levels changed proportionately. The number of cases in each group does not yet permit statistical evaluation.

SUMMARY AND CONCLUSIONS

A wide range of uropepsin and plasma pepsinogen values in normal subjects was found. Moreover, in a given individual there was a considerable fluctuation from day to day. Correlation between uropepsin and plasma pepsinogen values and the gastric acidity was poor. The results in ulcer patients were not constant, although high values were found in peptic ulcer with obstruction or bleeding and low values after gastrectomy. Following surgical trauma there were rather wide fluctuations of values with a tendency toward a decrease in pepsinogen production on successive postoperative days. Some drugs caused a marked effect, partly stimulating, partly inhibiting, but here again no uniformity was observed.

It is concluded that the plasma pepsinogen level (more conveniently determined than uropepsin) is of limited value as a diagnostic adjunct to conventional procedures in the diagnosis of conditions associated with hyper or hyposecretion of the gastric mucosa. While not closely related, there does exist a rough parallelism between plasma pepsinogen and gastric acidity at the extremes of the scale: that is, in patients with marked hyperacidity (complications of peptic ulcer) the plasma pepsinogen level is usually elevated and in patients with low or absent acidity (gastric resection, cancer and pernicious anemia) the pepsinogen level is usually diminished. In cases where examination by barium study, gastric analysis or gastroscopy is not helpful, as in hemorrhage, the plasma pepsinogen level may be of assistance in differentiating between esophageal and gastric lesions.

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INCREASE IN DIGESTIVE ACTION OF HUMAN GASTRIC JUICE FOLLOWING MAJOR SURGICAL PROCEDURES*

JOHN I. PERRY, JR. IARI C. YONIHURO PO MYAI YA, HARIAN D.
ROOT AND OWIN H. WANCINSSTEIN

Ulcers of the upper gastrointestinal tract first appearing after surgical procedures are serious complications; hemorrhage and perforation often accompany such lesions and the mortality attending them is high.¹ The data to be reported here indicate that increases in the concentration of pepsin occur in the gastric juice of patients in the early postoperative period and that these increments are such as to be associated with a digestive capacity of the juice for living tissues such as that seen with gastric secretion obtained from known duodenal ulcer patients. In addition, an increase in the hydrochloric acid content of the gastric juice, but to a less significant degree, occurs. This increase in the concentration of pepsin and possibly of acid would seem to be of fundamental importance in the genesis of those ulcers occurring in the post surgical period.

METHOD

Gastric secretions were collected from fasting patients by intubating gastric tube attached to suction for the 8 hr period from 11 p.m. to 7 a.m. Only patients who were to undergo major extragastric surgical procedures were chosen. A collection was made prior to operation and again within the first 96 hr following the operative procedure. Gastric secretions were collected over ice and were analyzed immediately or stored below 0°C until used. The volume of each specimen was measured and a sample removed for determination of pH, HCl, and pepsin. Free HCl was determined by titration with 1 N NaOH, pH was measured by the Northrup and Leeds glass electrode, and pepsin was determined by the hemoglobin substrate method of Anson² and expressed as tyrosine units per ml. of gastric juice. The remaining gastric juice was adjusted to pH 1.6-1.7 with hydrochloric acid to provide an optimum medium for peptic activity and used to perfuse the esoph-

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Fig. 1

agus of a living anesthetized cat for a period of 2 hr as previously described.² The animal was then sacrificed and the esophagus examined grossly and microscopically. The degree of digestion of the esophagus produced was graded as follows: *Grade 0*: No injury or only hyperemia of the esophageal mucosa; *Grade 1*: Erosion of the mucosal epithelium; *Grade 2*: Digestion into the submucosa; *Grade 3*: Digestion into the muscular layers of the esophagus; *Grade 4*: Impending perforation; *Grade 5*: Actual perforation during the perfusion period. Figure 1 shows minimal (*Grade 0*) and maximal (*Grade 5*) degrees of esophageal digestion produced. Statistical significance of mean values obtained was tested by use of the *t* test.⁴

RESULTS

For purposes of comparison the results obtained by studying in the same manner the gastric secretions of a group of control patients, duodenal ulcer patients and a large group of postoperative patients are shown in Tables 1 and 2.³ The pepsin concentration was significantly higher in both the duodenal ulcer group and the postoperative group as compared with controls.

Table 1 8 Hour Night Secretion

	TOTAL NO PATIENTS	FREE HCl MEAN	PEPSIN MEAN	pH (2.0 OR BELOW)
Control	35	2.87 mEq	1.0 u/cc	16%
Postoperative	61	7.60 mEq	1.4 u/cc	29%
Duodenal Ulcer	18	20.90 mEq	1.7 u/cc	33%

Table 2 Perfusion of Cat Esophagus with Human Gastric Juice pH 1.6-1.7

	TOTAL NUMBER OF PATIENTS	GRADE OF DIGESTION					
		0	1	2	3	4	5
Control	32	5	19	5	1	1	1
Postoperative	54	4	22	14	3	1	10
Duodenal Ulcer	18	1	3	1	3	5	5

Free acid concentration was significantly greater and the mean pH significantly lower in the duodenal ulcer group as well but not in the postoperative group as compared with controls

Table 2 shows the digestion of the cat esophagus produced by the gastric secretions of patients in these 3 groups. The necrotizing effects on the cat esophagus of the gastric secretions of both duodenal ulcer patients and patients in the postoperative period were significantly greater than that produced by the gastric secretions obtained from control patients. Only 10 per cent of specimens obtained from control patients caused grades 3 to 5 digestion of the cat esophagus, while 72 per cent of specimens from known duodenal ulcer patients and 26 per cent of specimens from patients in the early postoperative period caused these severe degrees of esophageal damage.

Gastric secretions collected from 25 patients both before and after operation were available for analysis and comparison. Tables 3 and 4 show mean values obtained for pepsin, free HCl, pH and volume preoperatively and after operation. The gastric secretions of 13 of these patients had no free hydrochloric acid present before surgery (Table 3). Pepsin values in this group were generally low and pH values reflected the absence of free acid. Following operation, mean values for pepsin and free acid were higher but the differences are not statistically significant. Mean pH and volume were also not significantly different from those preoperatively. In 12 patients free

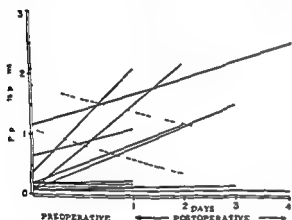
Table 3 8 Hour Night Secretion Patients with No Free Hydrochloric Acid in Gastric Juice Before Operation (13 Patients)

	PREOPERATIVE		POSTOPERATIVE		
	MEAN	RANGE	MEAN	RANGE	
Pepsin	49 u/cc	1-18 u/cc	10 u/cc	1-25 u/cc	9 increased 2 decreased 2 unchanged
Free HCl	0	0	5.59 mEq	0-41.47 mEq	4 of 13 had free HCl after operation
pH	4.8 (0 patients)	2.7-5.5 pH 2.0 or lower	4.3 (2 patients)	1.5-6.7 pH 2.0 or lower	
Volume	306 cc	10-800 cc	282 cc	60-740 cc	

Table 4 8 Hour Night Secretion Patients with Free Hydrochloric Acid in Gastric Juice Before Operation (12 Patients)

	PREOPERATIVE		POSTOPERATIVE		
	MEAN	RANGE	MEAN	RANGE	
Pepsin	1.87 u/cc	3-37 u/cc	2.8 u/cc	1-50 u/cc	10 increased 2 decreased
Free HCl	9.37 mEq	2.5-19.0 mEq	14.48 mEq	0-40.80 mEq	8 increased 4 decreased
pH	2.1 (6 patients)	1.4-3.0 pH 2.0 or lower	2.5 (6 patients)	1.5-5.3 pH 2.0 or lower	
Volume	250 cc	10-630 cc	415 cc	60-1090 cc	

hydrochloric acid was present in the gastric juice obtained before operation. Mean values obtained before and after surgery for pepsin free acid pH and volume are shown in Table 1. Pepsin values were generally higher before operation than in the group with no free acid and tended to parallel the amount of free acid present. Following surgery pepsin concentrations rose to even higher values and the increase over preoperative values is statistically significant. The mean value for free acid after operation is higher but the difference in means as compared with the preoperative value is not significant. Mean pH and volume values were not significantly different in the postoperative period as compared with preoperative controls. Figures 2 and 3 show graphically the changes in pepsin concentration occurring following operation in the 2 groups. Figure 2 indicates that in the group with no free acid initially several of the patients show no change in pepsin concentration after operation and in 2 an actual decrease in concentration occurred. In the remainder increase in concentration occurred. In the group with free acid (Fig 3) the tendency for higher pepsin values initially and greater increases in pepsin after surgery is shown. Two of 12 of these patients showed a decrease in pepsin concentration after operation and in the remainder a definite increase occurred.



CHANGES IN CONCENTRATION OF GASTRIC PEPSIN FOLLOWING OPERATION. PATIENTS WITH NO FREE HYDROCHLORIC ACID IN THE GASTRIC JUICE INITIALLY

Fig 2

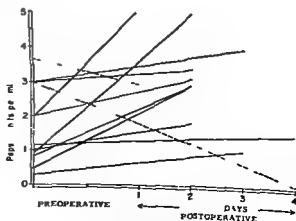


Fig 3

CHANGES IN THE CONCENTRATION OF GASTRIC PEPSIN FOLLOWING OPERATION. PATIENTS WITH FREE HYDROCHLORIC ACID IN THE GASTRIC JUICE INITIALLY

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Table 3 8 Hour Night Secretion Patients with No Free Hydrochloric Acid in Gastric Juice Before Operation (13 Patients)

	PREOPERATIVE		POSTOPERATIVE		
	MEAN	RANGE	MEAN	RANGE	
Pepsin	49 u/cc	1-18 u/cc.	10 u/cc.	1-2.5 u/cc	9 increased 2 decreased 2 unchanged
Free HCl	0	0	5.59 mEq	0-41.47 mEq	4 of 13 had free HCl after operation
pH	4.8 (0 patients pH 2.0 or lower)	2.7-5.5 (2 patients pH 2.0 or lower)	4.3 (2 patients pH 2.0 or lower)	1.5-6.7 (2 patients pH 2.0 or lower)	
Volume	306 cc	10-800 cc.	282 cc	60-740 cc	

Table 4 8 Hour Night Secretion Patients with Free Hydrochloric Acid in Gastric Juice Before Operation (12 Patients)

	PREOPERATIVE		POSTOPERATIVE		
	MEAN	RANGE	MEAN	RANGE	
Pepsin	1.87 u/cc.	.3-3.7 u/cc.	.28 u/cc	1-5.0 u/cc	10 increased 2 decreased
Free HCl	9.57 mEq	.2-19.0 mEq	14.48 mEq	0-40.80 mEq	8 increased 4 decreased
pH	2.1 (6 patients pH 2.0 or lower)	1.4-3.0 (6 patients pH 2.0 or lower)	2.5 (6 patients pH 2.0 or lower)	1.5-5.3 (6 patients pH 2.0 or lower)	
Volume	250 cc.	10-630 cc	415 cc.	60-1090 cc	

THE SECRETORY LIFE OF GASTRIC TRANSSECTION*

G. I. MOUNTAIN, J. H. LANDOR, J. D. MCCARTHY, P. V. HARPER
AND L. R. DRAGSTEDT

The occasional failure of transabdominal supradiaphragmatic vagotomy in ulcer therapy is usually due to incomplete interruption of the nerves as shown by physiologic tests. Dragstedt and Camp¹ stated that incomplete vagotomy exists in approximately 5 per cent to 10 per cent of cases. Oberhelman and Dragstedt² reported on a series of 187 patients treated by vagotomy and gastroenterostomy of which 28 subsequently developed stomal ulcers. Of these 28 recurrent ulcer cases, 11 had some intact vagal fibers as evidenced by a positive gastric secretory response to insulin hypoglycemia. This failure to attain a complete vagotomy in a small percentage of cases may be explained by the inconstant arrangement of vagal fibers along the lower esophagus.³ As it was felt that a more certain interruption might be accomplished by a high transection of the stomach with reanastomosis, the effect of this procedure on gastric acid secretion was investigated.

METHOD

Total isolated stomach pouches were prepared by the abdominal route in 8 female mongrel dogs with restoration of gastrointestinal continuity by end-to-end gastro-duodenostomy using the method described by Dragstedt *et al.*⁴ Stainless steel cannulae were placed in the isolated stomach just proximal to the antral fundic junction on the greater curvature side being brought to the outside through a stab wound in the abdominal wall. Seven of these pouches had good vagal innervation as evidenced by consistently positive responses to insulin hypoglycemia (10 units crystalline insulin intravenously in fasting state) on repeated testing. The eighth dog (D 212) had such poor response that it was considered essentially vagally denervated. After recovery a standard diet of 600 gm horsemeat, 500 cc milk and 800 cc water was given daily with electrolyte balance being maintained by the addition of sodium chloride. When the animals were consuming the full diet, rubber football bladders were attached to the cannulae by means of metal adapters and the gastric secretions were collected and measured every 24 hours. A 1 cc aliquot of each 24 hr specimen was titrated for its free acid content using 0.1 N NaOH with dimethylaminobenzene (Toepfer's reagent) as the indicator. Daily output of HCl in milliequivalents was then calculated by multiplying the volume secreted by the acidity in clinical units.

After a control period varying from 48 to 134 days the upper part of the stomach pouch was completely transected along with all vessels and nerves. This line of transection was carried medially across the lesser omentum. After ligating the vessels with silk, the 2 cut edges were anastomosed in their original position using 2 layers of #00 chromic cat gut. In half of the animals (D 212, 225, 859, 905) the transection was made across a line separating the upper one fifth from the lower four fifths of the stomach. In the remaining

*From the Department of Surgery of The University of Chicago. Aided by grants from the Division of Research Grants and Fellowship of the National Institutes of Health, United States Public Health Service and the Douglas Smith Foundation for Medical Research of The University of Chicago.

Table 5. *Perfusion of Cat Esophagus with Human Gastric Juice pH 1.6-1.7*

	NUMBER OF PATIENTS	DEGREE OF DIGESTION				
		0	1	2	3	4
Preoperative	17	3	11	2	1	
Postoperative	20		9	4	1	1

When perfusion of the cat esophagus to measure the digesting effect of the gastric juice on living tissue was carried out using specimens obtained preoperatively and postoperatively the results shown in Table 5 were obtained. Significantly greater degrees of digestion were produced by the gastric secretion obtained in the postoperative period than by those specimens obtained preoperatively. Both the gastric secretions from patients with free hydrochloric acid present before operation and those with no free acid showed statistically significant increases in esophageal digestion with gastric juice obtained postoperatively.

DISCUSSION

Gray and associates⁴ have demonstrated increases in gastric acid and pepsin and increased excretion of uropepsin to levels seen in duodenal ulcer patients in association with acute stress situations and have produced similar changes by the administration of ACTH or cortisone to patients and animals. Although significant increases in HCl in the gastric juice of postoperative patients could not be demonstrated in our studies many specimens had sufficient free acid present to maintain the pH at 2.0 or below a figure well in the activity range for pepsin and it is in this group with higher acid values that the greatest postoperative increases in gastric pepsin occur. These changes in the direction of those observed for gastric secretions obtained from duodenal ulcer patients offer in part at least an explanation for the mechanism of occurrence of postoperative ulcers.

SUMMARY

There occurs a significant increase in the concentration of pepsin in the gastric juice of many patients following operation. Increases in acid concentration are less definite. Associated with this change is an augmented capacity of the juice to digest living tissue. This increased peptic activity of the gastric juice is probably of fundamental importance in the genesis of postoperative ulcers.

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Table 1 Effect of High Cautic Transection and Subsequent Bilateral Transthoracic Vagotomy on Gastric Secretions in Dogs with Total Stomach Pouches

DOG NO.	INACIN TEST	TOTAL CAUSTIC FORCII			FOLLOWING TRANSECTION			PER CENT DECREASE IN ACID SECRETION	AFTER TRANSTHORACIC VAGOTOMY	
		NO. OF 24 HR. COLLECTIONS	MEAN VOL.	MEAN pH	NO. OF 24 HR. COLLECTIONS	MEAN VOL.	MEAN pH		NO. OF 24 HR. COLLECTIONS	MEAN pH
213	±	64	366	95.3±13.2	61	271	26.1±1.29	25	79	30.7±11.4
221	++++	48	724	83.4±21.5	61	416	23.2±10.1	70	74	36±8.1
859	++++	194	346	30.0±9.6	125	220	12.1±.8	60	64	23.0±10.5
905	++++	89	386	31.7±7.9	113	211	12.5±5.6	61	66	17.8±6.6
218	++++	54	515	51.6±17.9	46	146	10.2±1.0	81	57	11.7±1.9
283	++++	53	569	31.3±7.0	45	199	16.1±2.6	53	30	16.0±5.0
613	+	50	280	29.7±8.5	89	200	16.9±5.1	43	63	16.0±1.1
219	++++	49	321	31.3±12.0	56	256	16.8±2.0	51	48	12.1±3.9

TRANSFECTED 12 CM FROM CARDIA

1/2 - 1/2

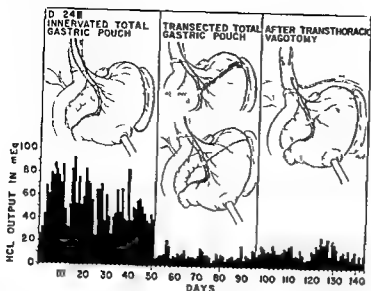


Fig. 1 The secretory effect of gastric transection

animals (D 248 283 643 719) the line of transection was approximately 2 cm from the cardia so that an extremely small amount of vagally innervated gastric mucosa remained. Insulin tests conducted after transection were consistently negative except for D 905 which exhibited a weakly positive delayed response. Daily secretions were again collected for periods of 45 days to 126 days following which all animals were subjected to bilateral transthoracic vagotomy. Following this third operative procedure daily secretions were again collected for 30 to 78 days and the experiment was concluded. All insulin tests made following this procedure were negative. All of the animals remained in excellent health throughout the experimental period.

RESULTS

The effect of gastric transection and subsequent transthoracic vagotomy on the daily 24 hr secretion of gastric juice in the 4 dogs studied is summarized in Table 1. All of the animals showed a highly consistent secretory response to gastric transection with an average reduction in the daily output of free hydrochloric acid of 55 per cent. In dog 212 apparently most of the vagal fibers were divided during construction of the pouch as there was no clear cut secretory response to insulin hypoglycemia. This animal had the least decrease in free acid (22 per cent) following gastric transection. Dog 643 had a moderate response to insulin hypoglycemia and similarly had less of a secretory decrease (43 per cent). The remaining animals exhibited a consistently strong secretory response to the insulin test and were thought to have good vagal innervation of their pouches.

Considering this group of 6 dogs alone following transection there was a 62 per cent decrease in the 24 hr output of free acid ranging from 18 mEq to 58 mEq. In general transection 2 cm from the cardia appears to have a slightly greater depressant effect on acid secretion but by analysis of variance this difference is not statistically significant due to the high interdog variability. Following gastric transection insulin tests were negative in all animals except for D 905 who demonstrated a delayed weakly positive secretory response. Following bilateral transthoracic vagotomy none of the animals demonstrated a statistically significant further reduction in the

tion in the upper innervated portion of the stomach at the time of trans-thoracic vagotomy may have masked the increase in secretion resulting from denervation of the small bowel with its assumed effect on increasing the intestinal phase. The efficacy of transection in reducing gastric secretions in this study seems correlated with the degree of initial vagal innervation although quantitative studies were not made to determine this.

SUMMARY AND CONCLUSIONS

Transection of the dog's stomach effectively interrupts vagal fibers as evidenced by a negative secretory response to insulin hypoglycemia. The results of this study we feel indicate that high gastric transection is as effective as transthoracic vagotomy in reducing acid secretion in the isolated stomach of the dog. While it is not suggested that this procedure be substituted for vagotomy in the routine clinical case, it might well be a useful alternate to gastrectomy in patients with incomplete vagotomy and recurrent ulcer who require surgery. The technical feasibility of this procedure in patients has been demonstrated in the management of bleeding gastric varices.

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secretion of free acid. However, in 2 (D 859-905) of the 3 animals in which a very high transection was carried out, transthoracic vagotomy resulted in an increase in the secretion of HCl. Insulin tests following this third procedure were once more negative except for D 905 which again demonstrated a delayed weak response.

DISCUSSION

The experiments reported were undertaken to determine the efficacy of vagotomy achieved by high transection of the stomach. We reasoned that such a procedure if it proved effective would offer a physiologic surgical approach of low mortality and morbidity to the patient who developed a stomal ulcer and in whom intact vagal fibers could be demonstrated by physiologic tests following transabdominal supra diaphragmatic vagotomy. The experimental data contained herein suggests that high gastric transection is as effective as transthoracic vagotomy in reducing acid secretion in the isolated stomach of the dog.

Transection of the esophagus might appear to be a more effective procedure as it would result in division of vagal fibers supplying the entire mucosa but this procedure entails the technically more demanding feat of esophageal anastomosis with the attendant risk of anastomotic leak and resultant mediastinitis. It might be argued that in contrast to the conventional method of resecting a small portion of the vagal trunks, gastric transection with reapproximation of the gastric walls might provide an ideal situation for regeneration of vagal fibers. This seems unlikely in view of the findings of Dragstedt, Woodward, and Camp⁶ who showed that simple crushing of the vagi without disturbing their continuity caused a profound decrease in gastric secretion in dogs which persisted for as long as three years.

A modification of this procedure has been used by Tanner⁷ and by Allen and Herd⁷ for the treatment of esophageal varices which had bled. Neither series is large enough nor has been followed long enough to permit definite conclusions but there were no subsequent episodes of bleeding in any of the patients followed by these authors. As a surgical treatment of esophageal varices the operation was introduced primarily as a method of interrupting the collateral circulation between the portal system and the esophageal veins but it possesses the added advantage of reducing the fasting gastric acidity thus decreasing the likelihood that erosion of vein walls by the corrosive action of gastric juice will occur.

In our present experiment the fact that transthoracic vagotomy increased the secretion in 2 of the animals previously subjected to transection of the fundus is a rather astonishing and as yet unexplained phenomenon. One possible explanation may be related to the fact that as shown by Ivy, Imt and McCarthy⁸ and by Gregory and Ivy⁹ the intestinal phase of gastric secretion in dogs is more pronounced when food does not pass rapidly through the intestine. Transthoracic vagotomy may by depriving the small bowel of its extrinsic parasympathetic innervation cause disturbances of intestinal motility which result in prolonged transit time with a resultant increase in the intestinal phase of gastric secretion. That this phenomenon was apparent in only two animals can be explained by assuming that transthoracic vagotomy had little direct effect on gastric secretion in these two. However, in the other animals the abolition of the cephalic phase of secre-

of the stomach in the same operation. A stainless steel cannula was placed in the pouch for the quantitative collection of gastric juice. In 2 animals a Heidenhain pouch was made as the first procedure in the usual way and after a suitable control period during which gastric juice was collected a total pancreatectomy was performed and the observations repeated. All animals were operated upon under general anesthesia and sterile technique. Quantitative 24 hr. collections of gastric juice were secured for long periods of time in all animals. The gastric juice was titrated against 0.1 normal solution of sodium hydroxide using Topfer's reagent as the indicator. The clinical units were multiplied by the volume expressed in liters and the product represents the excretion of hydrochloric acid in mEq. Postmortem examinations were made in all cases. Sections of the liver were taken and a careful inspection of the gastrointestinal tract was done. All animals were fed a diet of horse meat bread milk sugar and raw pancreas. Insulin was used as required. 3 to 6 gm. of lipocin were given orally each day.

The results are summarized in Tables 1 and 2. One of the dogs died of pneumonia 7 days after the operation and the data from this animal are excluded. Dog D 139 in which a Heidenhain pouch and pancreatectomy were performed at the same operation survived for 58 days dying eventually of pneumonia. Thirty eight 24 hr. collections of gastric secretion were obtained in this animal and the average daily output of hydrochloric acid was 55.5 mEq. Dog D 158 survived for 57 days and no explanation for death was found at autopsy except profound cachexia. Twenty 24 hr. collections of gastric juice were secured in this animal and the average daily output of hydrochloric acid was 38.9 mEq. In both of these animals the daily output of acid increased progressively from the time of operation until shortly before the animal died. In dog D 180 64 daily collections of gastric juice from the Heidenhain pouch were made before pancreatectomy was performed. The average daily output of acid was 55.1 mEq. Seventeen daily collections of gastric juice were made after the pancreatectomy and the average output of hydrochloric acid increased to 75.4 mEq. In the second animal D 181 65

Table 1 Gastric Secretion from Heidenhain Pouch in Dogs with Total Pancreatectomy

DOG NO.	NO. OF 24 HR. COLLECTIONS OF GASTRIC JUICE	DAILY OUTPUT OF HCl IN mEq
D 139	38	55.5 ± 13.4
D 158	20	38.9 ± 4.8

Table 2 The Effect of Pancreatectomy on Gastric Secretion from Heidenhain Pouch

DOG NO.	AVERAGE DAILY OUTPUT OF HCl IN mEq			
	NO. OF 24 HR. COLLECTIONS	BEFORE PANCREATECTOMY HCl	NO. OF 24 HR. COLLECTIONS	AFTER PANCREATECTOMY HCl
D 180	64	55.1 ± 2.4	17	75.4 ± 11.4
D 181	65	68.9 ± 28.4	18	65.1 ± 26.6

EFFECTS OF TOTAL PANCREATECTOMY ON GASTRIC SECRETION*

JAI ME BARCENA, JOSE L. BRAVO CIARENCI M. BAUCH
CLIFTON F. MOUNTAIN AND LESTER R. DRAGSTEDT

There are several observations suggesting a relationship between pancreatic function, gastric secretion and peptic ulcer formation. Since the original paper of Mann and Williamson¹ attention has been called to the protective effect of external pancreatic secretion and of bile in the experimental production of peptic ulcer. This beneficial effect has been attributed to the neutralizing properties of the alkaline secretions on the hydrochloric acid of the gastric juice. Owens² has called attention to the clinical importance of this neutralizing property in patients who have had a pancreatoduodenectomy.

Other observations have suggested the possible existence of humoral pancreatic factors which might produce or facilitate the production of peptic ulcers. Dragstedt³ in 1942 reported that whereas the incidence of duodenal ulcer in dogs with total external pancreatic fistulae approximates 100 per cent only 1 per cent to 3 per cent of dogs developed duodenal ulcers following total pancreatectomy. In both cases, pancreatic juice was absent from the gastrointestinal tract. In dogs with ligated pancreatic ducts and atrophy of the pancreatic parenchyma duodenal ulcers appeared in 29 per cent. Here again, pancreatic juice is absent from the gastrointestinal tract. Poth⁴ studied the frequency and severity of ulcer formation in pancreatectomized and total pancreatic fistulae dogs when treated with daily injections of histamine in beeswax and concluded that the presence of external pancreatic secretion was a very important factor in the prevention of this type of experimental ulcer. The same author has suggested the possibility that insulin or some hyperglycemic factor in the pancreas may play a very important role in ulcer production.^{5, 6}

Forty and Barret of England⁷ and Zollinger and Ellison in this country⁸ have called attention to patients with recurrent atypical progressive peptic ulcers associated with hyperacidity and hypersecretion which were not controlled by vagotomy and extensive gastric resection. These patients had islet cell tumors of the pancreas but in no case were there symptoms suggestive of or laboratory results compatible with hyperinsulinism. The hypersecretion of gastric juice was attributed to the presence of the islet cell tumor. The finding of a hyperglycemic factor in the serum of one of these patients suggested the possibility that these tumors may produce this factor and in turn stimulate gastric secretion.

The present experiments were devised to study the effect of total pancreatectomy on the secretions of gastric juice from Heidenhain pouches in dogs.

METHOD

In the first group of 3 female mongrel dogs a total pancreatectomy was performed and a Heidenhain pouch constructed from the greater curvature

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THE EFFECT OF ANASTOMOSIS OF THE PANCREATIC DUCT TO THE GASTRIC ANTRUM ON THE PRODUCTION OF EXPERIMENTAL PEPTIC ULCER*

JOHN R. BROOKS

It is a clinical fact that peptic ulceration of the second portion of the duodenum just distal to the entrance of bile and pancreatic secretions is rare. Experimental data suggest that the high alkalinity of these secretions is capable of buffering gastric acidity and thereby prevents mucosal ulceration. The Mann-Williamson¹ preparation by diverting these secretions removes this buffering protection and ulceration occurs. The work of Poth², Dragstedt³ and others revealed that duodenal ulceration is apt to occur in dogs following the experimental production of a pancreatic fistula or following ligation of the pancreatic ducts but that total pancreatectomy (removal of internal as well as external secretion) does not have the same ulcerogenic action. This work suggests that there is some acid stimulating factor, probably endocrine within the pancreas with which the alkalinity of external pancreatic secretion competes.

Dragstedt's work with intral and Heidenhain pouches^{1, 4} has shown that reflux of alkaline secretions or puddling of substances of high pH in the antrum of the stomach acts as a stimulus to gastric hydrochloric acid production and that substances of low pH have a reverse effect.

Although our knowledge of the role of external and internal pancreatic secretion in the control of gastric secretion is still not clear it appears that the pancreas may have, indirectly or directly, both stimulating and buffering actions.

It is the purpose of this experimental work now in its early stages to further investigate the part played by external pancreatic secretion and bile in the prevention of ulcer formation and to attempt to clarify the effect of reflux of alkaline secretions (pancreatic or biliary) into the antrum.

METHOD

Twenty-four adult mongrel dogs (male and female) have been operated upon. The animals were anesthetized with nembutal intravenously. Mann-Williamson procedures were carried out in all cases. The duodeno-ileal anastomosis was placed within 24 in. of the cecum in each case. Postoperatively the animals were fed Ken-L Ration and milk without bile salt or pancreatic substitution. The animals were reexplored in 4 to 8 wks. In some instances ulceration had not occurred and a later reexploration was necessary to await ulcer formation. All ulcers were proven by laparotomy and duodenotomy.

RESULTS

Four animals died at varying times (2 to 3 wks) in the postoperative period. 2 of distemper and 2 of causes unexplainable at postmortem study. All of these animals were thin and emaciated at death but none had evidence of ulcer formation.

Two animals developed Mann-Williamson ulcers which perforated and resulted in death before a second operation could be carried out.

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daily collections of gastric juice were secured before pancreatectomy. The average daily output of hydrochloric acid was 68.9 mEq. After pancreatectomy 18 daily collections of gastric juice were secured and the average daily output of hydrochloric acid was 65.1 mEq or approximately the same as before the pancreatectomy.

DISCUSSION

It is clear from these experiments that complete removal of the pancreas in dogs does not abolish or significantly reduce the secretion of gastric juice from the Heidenhain pouch. Total pancreatectomy thus does not abolish the humoral phase of gastric secretion. The marked increase in secretion observed in dog D 180 after pancreatectomy may be significant.

CONCLUSIONS

- 1 Total pancreatectomy does not abolish the secretion of gastric juice from a Heidenhain pouch in the dog.
- 2 Total pancreatectomy may produce a significant increase in the secretion of gastric juice from the Heidenhain pouch.
- 3 The intrinsic phase of gastric secretion is little affected by pancreatic function.

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which time the injections were discontinued. This dog is still alive and healthy 8 mo after pancreatic duct anastomosis. One other dog was given histamine 1 mon following a Mann-Williamson preparation. No pancreatic duct anastomosis was done. This animal died in 1 day of perforation and necrosis of the first portion of the jejunum just distal to the gastrojejunostomy.

C Common Duct Anastomosis to the Antrum (Choledochoantrostomy)
Two animals with Mann-Williamson ulcers were operated upon and anastomosis of the common duct carried out in a manner identical to that with the pancreatic duct. The pancreatic secretions continued to drain into the lower ileum. One of these animals showed complete ulcer disappearance 1 wk after anastomosis. One showed significant decrease in ulcer size to signify healing.

One animal had a Mann-Williamson procedure and choledochantrostomy carried out at one sitting. This animal had not developed the typical Mann-Williamson ulcer at the time of laparotomy 8 wks later.

DISCUSSION AND CONCLUSIONS

This investigative work is in its early stages and for this reason many of its ramifications have not yet been explored.

Anastomosis of the major pancreatic duct to the antrum of the stomach above a Mann-Williamson ulcer results in healing of this ulcer so long as the anastomosis remains patent. The constant stimulus to gastric secretion of the presence of highly alkaline pancreatic secretion in the antrum is apparently not sufficient to overcome the strong acid neutralizing action of pancreatic bicarbonate and for this reason healing of the ulcer occurs.

The number of animals in the choledochantrostomy series is small but the results suggest that bile delivered to the gastric antrum above a Mann-Williamson ulcer can also produce healing of such an ulcer.

Healing of these experimental ulcers following bile and pancreatic duct anastomosis seems most easily explainable on the basis of constancy of alkalinity. In the antrum as noted above such alkalinity may have two effects: one to set off the antral phase of gastric secretion, the other to act as a hydrochloric acid buffer. The addition of histamine to the experimental preparation upsets the balance and results in greater hydrochloric acid production and ulcer formation. Experiments are now under way to investigate the effect upon hydrochloric acid secretion from a Heidenhain pouch of anastomosing the pancreatic duct to a previously constructed antral pouch. Experimental evidence suggests that basal Heidenhain pouch secretory levels will rise as alkaline pancreatic secretion enters the antral pouch.

Clinically we have occasion to take advantage of this constancy of alkalinity in patients with duodenal ulcer disease. Medical regimens for ulcer have been directed for years at the maintenance of duodenal neutrality. If that portion of the duodenum just distal to the entrance of alkaline pancreatic and biliary secretions is excessively well protected against acid peptic digestion then there would be clinical reason to consider anastomosing the gastric stump to the second portion of the duodenum just distal to the ampulla of Vater following gastrectomy for duodenal ulcer. A more nearly normal reconstitution of the alimentary tract would result and the ulcer rate could be expected to be extremely low.

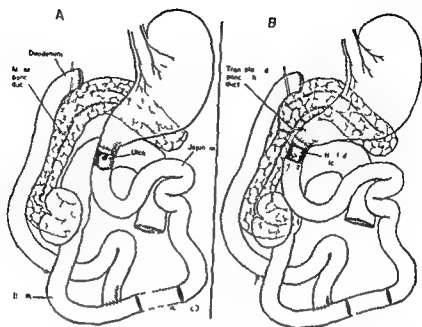


Fig 1

Seven animals recently operated upon have not yet had time to develop Mann-Williamson ulcers.

A Pancreatic Duct Anastomosis to the Antrum Ten animals developed typical Mann-Williamson ulcers in the first portion of the jejunum within 1 to 8 wks. Some of these animals explored at 4 wks had no ulcer only to develop an ulcer 3 to 4 wks later. In only 1 case was no ulcer apparent even 11 wks after the Mann-Williamson operation. All animals lost weight. The development of an ulcer was usually associated with inability to eat, more weight loss and general malaise.

Six of these animals with ulcers then underwent anastomosis of the major pancreatic duct to the antrum as shown in Figure 1. The anastomosis was placed on the lesser curvature, 1 to 1½ in above the gastrojejunostomy. Stomach on the superior surface of the antrum. An inner row of 5 0 silk was used to approximate the pancreatic duct to the antral mucosa. Over this a second row of fine cotton was used to telescope the antral muscularis and serosa around the duct to the pancreatic capsule. A polyethylene tube was used as a strut.

In 5 of these animals complete healing of the Mann-Williamson ulcer was noted at exploration and duodenotomy 4 wks later. In each case the pancreatic duct anastomosis was checked for patency and function by probing through a separate gastrotomy incision.

In 1 animal at exploration 4 wks after pancreatic duct anastomosis it was apparent that the original ulcer had enlarged 3 to 4 times. Gastrotomy revealed the pancreatic duct anastomosis to be occluded by scar.

B Pancreatic Duct Anastomosis plus Histamine Three animals whose Mann-Williamson ulcers healed following pancreatocolectomy remained healthy for 17 and 8 wks respectively at which time they were given histamine in beswax daily according to the method of Hay. Two of these animals developed perforated jejunal ulcers within 3 to 5 days of the onset of histamine injection. The other animal survived 1 wk of histamine at

which time the injections were discontinued. This dog is still alive and healthy 8 mo. after pancreatic duct anastomosis. One other dog was given histamine 1 mon. following a Mann-Williamson preparation. No pancreatic duct anastomosis was done. This animal died in 4 days of perforation and necrosis of the first portion of the jejunum just distal to the gastrojejunostomy.

C. Common Duct Anastomosis to the Antrum (Choledochointrostomy). Two animals with Mann-Williamson ulcers were operated upon and anastomosis of the common duct carried out in a manner identical to that with the pancreatic duct. The pancreatic secretions continued to drain into the lower ileum. One of these animals showed complete ulcer disappearance 4 wks. after anastomosis. One showed significant decrease in ulcer size to signify healing.

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HEIDENHAIN POUCH SECRETORY RESPONSE TO GASTROJEJUNOSTOMY AS AFFECTED BY POSITION ON THE STOMACH AND VARIATION IN STOMAL DIAMETER*

JOHN A. STEVENSON JOHN E. JESSEPH THOMAS W. JONES
AND HENRY N. HARKINS

Previous investigators^{1, 2} reported no stimulatory effect on Heidenhain pouch secretion following low antral gastrojejunostomy utilizing a narrow stoma (less than 3 cm in diameter). At the same time, in our laboratory³ utilizing a larger number of animals we found a definite stimulatory response utilizing a wider stoma (4 cm in diameter).

Because clinical advice for performing a low gastrojejunostomy in patients is based on the former but disputed observation we thought it advisable to repeat the experiment in an attempt to settle the problem in a definitive manner.

After careful study of all the factors involved the only common variant in the experimental preparations which might account for the different results was found to be the size of the gastrojejunostomy stoma. With this in mind the experiment was repeated with controlled variation of this factor.

METHOD

A group of 7 mongrel dogs of both sexes and weighing between 8 to 15 kg was used. Environmental conditions were carefully controlled as to kennel temperature, time of feeding and time of collection of Heidenhain secretions. A Heidenhain pouch was constructed in each dog and standardized in the routine manner previously reported.^{3, 4, 5}

The results are quantitatively expressed in mEq HCl secreted for 24 hr and represent an average of 30 days collections. After adequate Heidenhain

pouch standardization a low intral intestinal isoperistaltic gastrojejunostomy was performed in all dogs. The jejunal anastomosis was made 10 cm distal to the ligament of Treitz and in the ileum 2 to 3 cm proximal to the pylorus. In each instance the procedure was the same except for the construction of the stoma which varied in size from 2 to 4 cm in diameter. Adequate time was allowed for the animal to recover from the operation before Heidenhain pouch secretions were again collected. After 30 consecutive 24 hr Heidenhain pouch collections had been made the animals were reoperated and the stoma size changed. All 2 cm diameter stomas were converted to 4 cm diameter and vice versa. The animals were again allowed to recover from the operative procedure before Heidenhain pouch collections were resumed. Collections were made for a period of 30 days in each animal after which the stoma was changed to its original diameter and another group of collections made. To terminate the experiment the gastrojejunostomy was taken down and a final period of Heidenhain pouch collections was made with normal bowel continuity restored.

In each instance stoma sizes were measured *in vivo* as accurately as possible with 2 cm and 4 cm diameter glass rods at the time of construction and at the time of conversion. Additional *in vivo* barium studies were made to determine the relative patency of the stomas and the main direction of flow.

RESULTS

There was no mortality in this group of experiments. The animals tolerated the multiple operative procedures well as evidenced by maintenance of body weight, appetite and general well being.

When compared to the standardized Heidenhain pouch secretions there was an average increase of 48.8 per cent in the secretions collected during the periods that the animals had a gastrojejunostomy with a 2 cm stoma. However, when the animals had a 4 cm diameter gastrojejunostomy stoma there was a greater increase in HCl secretion by the Heidenhain pouch averaging 173.5 per cent. When the gastrojejunostomy was taken down and the Heidenhain pouch restandardized there was a 40.0 per cent increase in HCl secretions over the pre-gastrojejunostomy level (Fig. 1).

Barium studies done in each interval demonstrated in every instance the stoma to be patent. In general when the animal had the 2 cm diameter gastrojejunostomy stoma there was a greater tendency for the barium to flow through the duodenum although some was seen to enter the jejunum directly. When the animal had a 4 cm diameter gastrojejunostomy stoma the flow of barium was directly into the jejunal loop with very little entering the duodenum (Fig. 2). Evaluation of intragastric regurgitation or circus movement was not possible.

SUMMARY

Under the conditions of this experimental study we found that there was only a moderate increase in Heidenhain pouch secretion following a low intral gastrojejunostomy if the stoma was constructed 2 cm in diameter. If however the gastrojejunostomy stoma was constructed 4 cm in diameter there was a marked such increase. The average increase in secretion of free HCl from the Heidenhain pouch when stimulated by 2 cm diameter gastro

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The results are quantitatively expressed in ml q HCl secreted for 24 hr and represent an average of 30 days collections. After adequate Heidenhain

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CONCLUSION

Under the conditions of this experiment stomal diameter in the case of a low gastrojejunostomy has a definite influence on the amount of Heidenhain pouch free HCl secretion.

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RELATIVE SUSCEPTIBILITY OF THE GASTROINTESTINAL TRACT TO EXPERIMENTAL ACUTE PEPTIC ULCERATION*

LOUIS F. PIZAK, JR., WALTER FRIED AND EDWARD R. WOODWARD

When an anastomotic channel is created between the stomach and small intestine (especially in patients with duodenal ulcer) peptic ulceration of the exposed intestinal mucosa is a discouragingly frequent occurrence. In the genesis of these stomal ulcers the following 4 factors have been considered important: 1) the persisting secretion of acid-peptic juice; 2) the sensitivity of the gut wall *per se* to acid-pepsin digestion; 3) the neutralizing ability of bile, pancreatic juice and succus entericus; 4) the protective activity of mucus. This paper is concerned with an investigation designed to study the relative sensitivity of the various segments of the gastrointestinal tract to peptic digestion.

Following subtotal gastrectomy with a Billroth II reconstitution of gastrointestinal continuity it has been commonly observed that as the stomach is anastomosed to progressively lower segments of the intestinal tract there is an increasing incidence of stomal ulcer. This observation has suggested to many workers that there is a gradient of susceptibility to ulceration. However, in a series of studies Merendino and co-workers^{1, 2, 4} found that the neutralizing capacity of the intestinal contents at various levels could ac-

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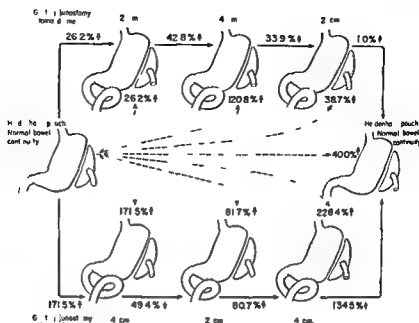


Fig 1 The sequence of operative procedures with resultant succeeding increases or decreases in free HCl secretion from Heidenhain pouches is shown. In the upper portion of the chart the sequence is shown when the first anastomosis was 2 cm in diameter while below the sequence is demonstrated when the first anastomosis was 4 cm in diameter. In all instances the final stage was a complete takedown of the anastomosis.

The solid lines represent average per cent increase (↑) or decrease (↓) of free HCl secretion produced by an actual operative procedure. The dotted lines indicate the per cent increase (↑) of each preparation as compared to the standardized control Heidenhain pouch with normal gastro intestinal continuity. The increases as compared to the control for the 2 cm stomas were 26.2, 38.7 and 81.7 per cent (average 48.8 per cent). The increases as compared to the control for the 4 cm stomas were 17.5, 49.4 and 228.4 per cent (average 173.5 per cent). The overall effect of multiple procedures following takedown of the anastomosis in the 7 dogs was a 400 per cent increase.

jejunostomy was 48.8 per cent. For a 4 cm stoma the similar average was 173.5 per cent or over 3 times as much. If the 100 per cent increase produced by multiple operations alone is considered this difference is even greater.



Fig 2(A) Barium study of typical preparation with 2 cm gastrojejunostomy stoma. Most of the barium goes out the pylorus only, a portion through the stoma. (B) Barium study of typical preparation with 4 cm gastrojejunostomy stoma. All of the barium goes through the stoma, none through the pylorus.

Table 1 *Histologically Determined Extent of Penetration of Ulcers in Various Regions of the Digestive Tract*

EXPERIMENT	REGION OF GASTROINTESTINAL TRACT							COLON
	ESOPHAGUS	ESOPHAGUS	STOMACH	STOMACH	DUODENUM	JEJUNUM	ILEUM	
A		4	1		1		1	
B			1			1	1	5
C		4	3	3		1	1	7
D	4	4	3	3	4	1	1	
E	4	4	1	2	3	1	1	5
F					2	2	2	4
G					5	5	5	5
H					5	5	5	5
I					3	3		

NUMBER	DEPTH OF EROSION OR ULCERATION
1	In the epithelium and lamina propria
2	Up to the muscularis mucosa
3	In the muscularis mucosa
4	Up to the submucosa or in it superficially
5	In the submucosa

compared not only grossly but also by microscopic study of serial sections of the ulcers with determination of the layers of gut penetrated at the point of maximal damage

RESULTS

The terminal results are summarized in Table 1

Esophagus The sequence of events and the terminal condition of the esophagus were the same in both areas selected for study. Ischemia was noted within a minute after the stream was started and the mucosa was penetrated within 5 min. The underlying layers thus exposed soon presented a glossy translucent appearance which did not change as the experiment progressed. Even after two hr. there was no further penetration of these layers by acid pepsin solution.

Stomach Of the 8 sites tested the 2 sites selected in the stomach were the most resistant to ulceration. The damage to the stomach mucosa appeared much later than did injury to the other sections of the gastrointestinal tract tested. The antral and fundic mucosa seemed to be about equally resistant to the acid pepsin solution.

Intestine The 3 areas of the small intestine investigated seemed equally susceptible to erosion. Within a fraction of a minute after beginning the streams the areas became ischemic and petechial hemorrhages appeared. The mucosal surface was rapidly eroded and after 15 to 40 min. (depending upon the experimental conditions) necrosis extended to the muscularis mucosae. This layer is relatively resistant to destruction and was not always penetrated

count for this apparent gradient of susceptibility and concluded that the inherent mucosal sensitivity is relatively unimportant in peptic ulceration.

Le Veen⁴ reported a method of producing acute damage to the gut with a constant fine stream of acid pepsin solution which washed away all neutralizing and neutralized substances and thus continuously exposed the selected area to fresh acid pepsin solution. By utilizing a modification of Le Veen's technique we have further investigated the question of the importance of tissue susceptibility *per se* in peptic ulceration of the gastrointestinal tract.

METHOD

Adult mongrel dogs under nembutal anesthesia were utilized as the experimental animal. The dogs were intubated and respiration was maintained by positive pressure anesthesia.

Artificial gastric juice was prepared at pH 1.2 and concentration of 4 to 5 mg pepsin per cc. The acid pepsin solution was warmed in a water bath at 37°C and maintained at this temperature throughout the experiment. Rubber tubing leading from the pepsin solution was passed through an electrically driven pump. Distal to this pump the stream was divided by a series of interposed glass Y tubes into multiple jets issuing from No. 22 gauge needles. The size of stream, rate of flow, and the pressure of the jet of fluid escaping from each needle were uniform for each experiment; the pressure used varied from 34 to 48 cm of water in the different experiments.

Eight areas of the gastrointestinal tract were selected for simultaneous exposure to the stream of peptic juice. They were:

- 1 Esophagus 7 cm distal to the cricoid cartilage
- 2 Esophagus 1-2 cm proximal to the cardiac sphincter
- 3 Fundus of the stomach midposterior wall
- 4 Pyloric antrum of the stomach posterior wall
- 5 Duodenum 10 cm distal to the pylorus
- 6 Jejunum 10 cm distal to the ligament of Treitz
- 7 Ileum 10 cm proximal to the ileocecal valve
- 8 Colon 10 cm distal to the ileocecal valve

Ten to 15 cm segments of gut containing the areas to be perfused were carefully ligated to preserve fully the blood supply and were slit open along the antimesenteric border. The mucosal surface was everted and the mesenteric border of the gut was exteriorized. The exposed tissue was positioned so as to maintain a constant minimal tension on it and to provide optimal drainage of juice from the proposed ulcer site. Drainage was provided either by gravity or by strategically placed suction equipment. The remainder of the abdominal contents was protected from the peptic solution by saline sponges. The needles through which the peptic solution passed were held in place 3 cm from the mucosa and at 45° angles to the exposed surface by burette clamps. The jets were all started at once and gross observations of the exposed sites were recorded as the experiment progressed. The average duration of the 9 experiments was approximately 2 hr; time varied from 45 min to 2½ hr. Adequate vascular supply to the immediate area of the lesion was ascertained at the conclusion of the experiments by noting considerable bleeding as the ulcer sites were excised for histological study. The dogs were then sacrificed. The relative degrees of ulceration in the various areas were

secretions contained in the gut. On the basis of our experiments we concluded that

1. The mucosa of the esophagus (both upper and lower) is least resistant to peptic ulceration but the damage does not penetrate further into the tissue even when exposure to the jet of peptic solution is greatly prolonged.

2. The antrum and fundus of the stomach are about equally resistant to ulceration; these are the most resistant parts of the gastrointestinal tract.

3. Punched-out ulcers form with essentially equal facility in the duodenum, jejunum and ileum in the absence of neutralizing secretions. These tissues are intermediate between the esophagus and stomach in their susceptibility to ulceration.

4. Although the epithelium and lamina propria of the small and large bowel are equally susceptible to erosion, the muscularis mucosa in the colon is more readily penetrated and hence the colon is more susceptible to true ulceration than the rest of the intestine.

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THE GENESIS OF INTESTINAL ATRESIA*

G. N. BARNARD

Since the time that Osiander recorded the first case of congenital occlusion of the intestine in 1797 many views as to the causation of this anomaly have been expressed. Most of these were based on isolated morbid anatomical findings or indirect experimental evidence and can be briefly summarized under 3 main headings: 1) *Developmental defects*. Under this heading are grouped the theories of Meckel, Bland-Sutton and Trudler who all attempted to explain the anomaly on some failure of correct development of the fetal gut. 2) *Fetal accidents*. Intra-abdominal catastrophes such as intussusceptions, volvuli, kinks, etc. were blamed by some for the atresia. 3) *Fetal disease*. Several investigators incriminated fetal disease such as peri-

*From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minn. Supported by Austen S. and Anne F. Cargill Fund for Surgical Research, Jav. and Rose Phillips Fund, Richard C. Lilly Fund.

within the duration of the experiment. The observations in the duodenum, jejunum, and ileum were consistently identical; data from the colon indicated that it was somewhat more susceptible to ulcer formation than small intestine. The mucosa was eroded down to the muscularis mucosa at the same rate in the small bowel; damage from this point on progressed more rapidly in the large intestine.

Control. As a control, 2 sites 5 cm apart in the jejunum were selected for subjection to a stream of heated (37°C) HCl solution at pH 1.2 and the acid pepsin solution used in the experiments. The conditions at each site were identical. No erosion occurred at the site that received only the acid, whereas the pepsin solution in the same time interval produced the typical acute ulceration noted above.

The correlation between the gross observations during the experiment and the histologic findings was remarkably close.

DISCUSSION

We desired to use a jet of fluid in this experiment so as to deliver a peptic solution of known activity to the selected area constantly and to wash away continually any mucus secreted by the gut. The ulcers produced did not result merely from the pressure of the system since a comparable acid control solution without pepsin was impotent when compared to the acid pepsin solution under the same conditions. The results indicate the degree of actual innate tissue resistance to ulceration in the various segments of the alimentary tract insofar as the protective effects of gut contents and mucosal secretions have been eliminated by our experimental method.

The finding that the esophageal mucosa is most susceptible to peptic digestion was expected on the basis of numerous other observations and investigations; it was surprising, however, to note that after erosion of the mucosa no further damage seemed to occur in the time intervals studied. The great degree of resistance on the part of the stomach was to be expected. Difficulties in maintaining equivalent amounts of tension during the experiment in the fundic and antral areas prohibited a precise conclusion concerning the relative susceptibilities in these regions. It can be said that there are no considerable differences in resistance in these parts of the stomach. The essentially identical degree of damage in the duodenum, jejunum, and ileum as the experiments progressed indicated that these areas of the small bowel are apparently equally susceptible to acute peptic ulceration. The colon usually ulcerated more rapidly than the small bowel; this seemed to be due to greater ease of penetration through the muscularis mucosae in the colon since the epithelium and lamina propria were eroded at the same rate in both the small and large gut. The facility of penetration of the muscularis mucosa in the colon may be due to the thinness of this layer as compared with that in the small intestine.

SUMMARY AND CONCLUSIONS

We have presented evidence of the relative susceptibility of the gastrointestinal tract in the dog to experimental acute peptic ulceration by subjecting selected areas of it to a stream of pepsin solution at pH 1.2. By our technique we have tried to examine the innate sensitivity of the tissue to ulceration at various levels by excluding the effects of neutralizing and protective



Fig 2 Autopsy findings in puppy born 10 days after intrauterine operation. Proximal bowel distended ending in bulbous tip area of atresia and collapsed distal bowel



Fig 3 Histomicrograph showing the fusion of distal and proximal blind ends with the formation of a septum

tion of the infarcted segment with at the most a complicating meconium peritonitis

The question may be asked. But how do we know that the vascular interference precedes the atresia and not atresia the vascular occlusion?

METHOD

Operations were done upon dogs in utero. In some experiments the mesenteric vessels which supply a short segment of small bowel were ligated. In other experiments the blood supply to a segment of ileum was interrupted by creating a strangulating obstruction. The mother dog was then allowed to go to term and the puppies operated upon were sacrificed within 24 hr after birth.

On post mortem examination of these puppies it was found that the infarcted or strangulated segment was in various stages of disintegration depending on how soon the puppy was born after the interference to the blood supply. In puppies born from 7 to 10 days after surgery in which the mesenteric vessels were ligated an anomaly of the gut identical with that found in infants with ileal atresia resulted. The bowel proximal to the infarcted segment was found to be distended, ending in a bulbous tip. The bowel distal to the infarcted area was normal but completely collapsed. There was a V-shaped defect in the mesentery and the infarcted bowel was entirely represented by a fibrous strand. (See Fig 2) In puppies born from 7 to 10 days after a segment of bowel was strangulated the same defect resulted, however in some of these puppies on account of the strangulation the proximal and

tonitis or intestinal ulceration. When all these theories are studied in more detail it becomes certain that one mechanism cannot explain all cases of congenital intestinal atresia.

Louw¹ reviewed 79 consecutive cases of atresia and severe stenosis in the newborn at the Hospital for Sick Children London and noted that certain features of the anomaly suggested that some atresias may be due to interference with the blood supply to a portion of fetal gut. These features include the following: 1) Anomalous vascular supply to the atretic portion by a V shaped defect in the mesentery. 2) Early necrosis of the proximal blind end before the tension in this area has risen to any extent. This occurred in 20 per cent of the cases and in half of these cases the infant was less than 48 hr old. 3) Postoperative atony and ileus in apparently healthy blind ends anastomosed to the distal bowel. Similar observations have been reported by other workers and the following cases studied at Groote Schuur Hospital Cape Town under Prof J H Louw* further strengthened our belief in the vascular hypothesis: (a) An infant born with aplasia of the descending and pelvic colons in whom there was complete absence of the inferior mesenteric artery. (b) An infant operated on for ileal atresia whose resected specimen clearly showed the remnant of an intussusception in the distal segment. (c) A newborn infant with intestinal obstruction whose ileum clearly showed the presence of a volvulus which was presumably responsible for the atresia (Fig 1). (d) An infant with intestinal atresia in whom at operation a congenital band had caused infarction of the bowel and was probably responsible for the atresia.

Although strangulation causes infarction of the bowel followed by gangrene, perforation and peritonitis in postnatal life the train of events are different in the sterile fetal bowel. Here strangulation results in the absorp-



Fig 1 Segment of ileum resected at operation showing atresia at the ileum and the presence of a volvulus.

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The Liver and Pancreas

NITROGEN BALANCE IN ANIMALS WITH BILIARY FISTULA*

LOREN J HUMPHREY

Persistent loss of bile is associated in most patients with a marked weight loss. The increase in fecal lipid excretion with a biliary fistula is well known as an important factor in the general debility of these patients. Protein metabolism has received less attention in investigations concerned with patients and animals with biliary fistula. In this paper nitrogen balance, plasma volume and circulating protein studies are reported in dogs with a biliary fistula.

METHOD

Mongrel dogs of both sexes varying in weight from 7 to 13 kg were used in this study. All dogs received 60 mg of morphine sulphate preoperatively and 100 mg/kg of sodium pentothal during surgery.

An internal biliary fistula was chosen for these studies in an attempt to avoid the cholangitis associated with external methods of bile diversion. The procedure was that of cholecystonephrostomy as described by Kapsinow, Engle and Harvey.¹ In this operation the gallbladder is anastomosed to the right renal pelvis through a transcortical incision in the kidney after ligation and section of the common bile duct.

All dogs were placed on a special diet containing 65 per cent fat, 20 per cent protein and 15 per cent carbohydrate and given 3.4 gm of protein/kg/day and 75 calories/kg/day. One control period (i.e. 1 wk) was obtained in the first 4 dogs and 3 control periods (i.e. 3 wks) were obtained in the last 3 dogs. During these control periods plasma volume, blood protein and nitrogen balance determinations were made.

Beginning with the third postoperative week successive 7 day periods were run with each dog being placed on the special diet.

The Evans Blue dye method was used in determining weekly plasma volumes. Total serum protein, serum albumin and serum globulin concentrations were determined weekly using the biuret method. Dietary and urinary nitrogen were determined by the Micro-Kjeldahl method. Since Heersma and Annegers² state that fecal nitrogen varies only with the fiber content of the diet in dogs with biliary fistula we determined only dietary and urinary nitrogen and used F as a correction factor for fecal nitrogen.

RESULTS

A total of 28 dogs were operated upon in this study. The operative mortality was 14.3 per cent. Eight dogs were used as a preliminary study to de-

*From the Department of Surgery, University of Illinois College of Medicine, Chicago, Ill. Aided by a grant from the Graduate School, University of Illinois. With the technical assistance of Mr. Everett Hoppe.

distal blind ends were pulled so close together that they fused and a septum resulted (Fig 3)

These successful experiments together with the other observations constitute sufficient proof that at least a proportion of intestinal atresia are due to a vascular accident causing infarction of a segment of a fetal bowel and absorption of the affected segment resulting in atresia. It also shows one mechanism by which a septum can result.

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The Liver and Pancreas

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METHOD

Mongrel dogs of both sexes varying in weight from 7 to 13 kg. were used in this study. All dogs received 60 mg. of morphine sulphate preoperatively and 22 mg./kg. of sodium pentothal during surgery.

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termine the most palatable diet and the minimum requirements for a positive nitrogen balance

Of the remaining 20 dogs 7 dogs fulfilled the criteria for inclusion in this series. The majority were excluded because of failure to ingest dietary nitrogen in amounts equivalent to that of their control period

All dogs lost weight rapidly and appeared quite cachectic. The average survival time of these 7 dogs was 8 wks and the average weight for these 7 dogs at 6 wks postoperatively was 28.4 per cent below the preoperative average

Data on plasma volume determinations shows a progressive decrease in total plasma volume averages postoperatively with an average decrease of 26.5 per cent 6 wks postoperatively below the average of control values. However serum protein levels at 6 wks postoperatively increased 17.4 per cent above control levels

At 6 wks postoperatively the serum albumin concentration was 31.7 per cent above the control level and the serum globulin concentration average was 12.5 per cent below the control level

Nitrogen balance determinations in all 7 dogs showed a more negative nitrogen balance after operation than in the control periods. The average preoperative urine nitrogen excretion on these dogs was 35.36 gm for each 7 day period. These same dogs postoperatively excreted an average of 45.63 gm of nitrogen in the urine for each 7 day period. This means that postoperatively on an average 10.27 gm of nitrogen/wk or 64.19 gm of protein/wk were lost in excess of control values. Biweekly nitrogen balance averages on all dogs and nitrogen balance averages for individual dogs are given in Figures 1 and 2

DISCUSSION

It is well known that the lack of bile in the intestinal tract causes a marked weight loss. This has been reported by Cole,³ Bissell,⁴ Colwell,⁵ and

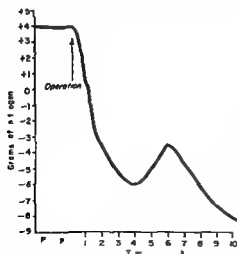


Fig. 1 Biweekly nitrogen balance averages for 7 dogs showing preoperative control level and levels for 10 postoperative wks following the production of an internal biliary fistula

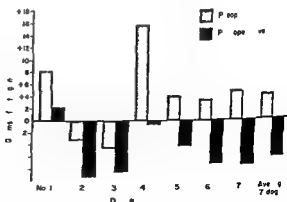


Fig. 2 Nitrogen balance averages for individual dog showing preoperative control levels and the average levels of 5 postoperative wks following the production of an internal biliary fistula

others. In this study there was severe weight loss leading to death from cachexia on the average within 8 wks.

Paralleling the marked weight loss in this study is a marked decrease in total plasma volume. It is also seen from this study that total serum protein concentrations rise progressively when bile is absent from the intestinal tract. This progressive rise is probably due to concentration of protein as plasma volume diminishes.

It is seen that in these dogs with biliary fistula there is a progressive increase in serum albumin concentration and a progressive decrease in serum globulin concentration beginning at the fourth week postoperatively. Since the half life of alpha globulin is much shorter than that for albumin it is possible that this is a result of starvation.

The primary purpose of this study was to determine the effect of diversion of bile from the intestinal tract on nitrogen balance. It is seen that the difference between control nitrogen balance averages and postoperative nitrogen balance averages is 10.27 gm. for a 7 day period. In terms of protein this means each dog lost 64.19 gm. more of protein each week postoperatively than was ingested. However from this study we cannot say whether the more negative nitrogen balance postoperatively resulted from a decrease in fat absorption and hence a loss of protein sparing action of fat, or whether the loss of bile from the hepatic circulation alters the metabolism of liver cells or assimilation of protein.

SUMMARY

An experiment has been conducted to determine the effect of the diversion of bile from the intestinal tract on nitrogen balance. A cholecystonephrostomy was performed on 7 dogs which had been studied preoperatively for control periods.

All dogs progressively lost weight and the average length of survival postoperatively was 8 wks. The decrease in total plasma volume paralleled this weight loss while total serum protein concentration increased slightly in all dogs postoperatively. At 1 wks postoperatively serum albumin concentration increased progressively and serum globulin concentration decreased progressively.

A more negative nitrogen balance was found to exist postoperatively in all the dogs with a biliary fistula in this series. In these dogs the difference between the control nitrogen balance average and the postoperative nitrogen balance average was 10.27 gm./wk.

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THE ROLE OF THE SHWARTZMAN REACTION IN THE PATHOGENESIS OF INFLAMMATORY LESIONS OF THE BILIARY TRACT*

STUART ROBERTS AND RICHARD WEBB JR

In studying the dermal Shwartzman reaction it appeared that this phenomenon of local tissue reactivity consisting of hemorrhagic necrosis with healing by fibrosis may be implicated in the occasional progressive inflammatory destruction of the common duct which has been described as obliterative cholangitis. Most surgeons¹ agree that the vast majority of strictures of the common duct result from trauma inflicted during operation yet in a small percentage of cases infection itself appears to be the primary factor.² In some instances the infection behaves in an unusual fashion progressing to the destruction of the duct converting it into a fibrous cord.³

The Shwartzman reaction has been elicited in many tissues and organs including skin, stomach, appendix, bowel and pancreas^{1,4} but it has not been applied to the pathogenesis of strictures of the common duct nor to severe inflammatory reactions occasionally seen in the biliary tract.

A brief description of the phenomenon and definition of terms are essential in order to fully understand the experiments. The Shwartzman reaction is a 2 stage reaction (Table I) in which the first event is sensitization of the desired tissue brought about by the local injection of culture filtrates or toxins of certain gram negative micro organisms; the second event is initiated by the intravenous injection of the same or other toxin (diluted) 24 hr later and consists of hemorrhagic necrosis of the sensitized tissue. The first injection is referred to as the sensitizing injection. The second injection 24 ways given intravenously 24 hr later is called the challenging injection.

METHOD

Toxin. Meningococcal toxin was prepared from agar washings of cultures 44 B strain of meningococcus obtained through the kindness of Dr Gregory Shwartzman, Mount Sinai Hospital, New York. The toxin was prepared according to the method of Shwartzman.⁵ Each batch of toxin was tested for potency immediately prior to use by an intradermal injection (25 cc) in

Table I: Elicitation of the Shwartzman Reaction†

STAGE ONE (SENSITIZATION)	
Route of Injection	Application of Methods
(1) Into parenchyma of tissue 1 cc	Into wall of common duct or gallbladder
(2) Application to surface of tissue 1 cc	Instillation into lumen of common duct
(3) Into vascular supply of organ 1 cc	Into cystic artery
INCUBATION PERIOD	
24 hr	
STAGE TWO (CHALLENGING)	
Intravenous Injection 1 cc	Into jugular vein

†Modified from Rostenberg.

*From the Department of Surgery, University of Illinois College of Medicine, Chicago, Illinois. With the technical assistance of Everett Hoppe and Ruth McGrath. Aided by a grant from the Graduate School, University of Illinois.

rabbits followed by an intravenous injection (2 cc of 1:10 dilution) of the same toxin 24 hr later. Potent toxin uniformly produced an area of dermal hemorrhagic necrosis 1×3 cm in diameter. The toxin was stored at 1°C and each batch of toxin was used within 3 wks.

Animals. Except for testing of potency of toxin in albino rabbits, adult male and female goats of domestic breeds weighing 20 to 62 kg were used throughout the experiments. The goats were lightly anesthetized with intravenous pentobarbital using an endotracheal tube. Dogs are not susceptible to the Shwartzman reaction.

Histologic Methods. As soon as the animals died or were sacrificed, tissues were fixed in 10 per cent formaldehyde solution. Sections were stained by the hematoxylin and eosin method.

Defunctionalization of the Common Duct. The distal portion (3 to 4 cm) of common duct was defunctionalized by placing a ligature around the common duct just distal to the point of entrance of the pancreatic duct into the common duct. Decompression of bile and pancreatic secretions was accomplished by cholecystoduodenostomy (Group 2).

RESULTS

The Shwartzman reaction was elicited in the common duct or gallbladder in 7 of the 16 goats utilized in the 3 groups.

Group 1. Injection Into Wall of Common Duct or Gallbladder. In this group the toxin was injected (sensitization) into the wall of the common duct or gallbladder of 11 goats followed by an intravenous (challenging) injection of toxin 24 hr later. There were positive results in 6 animals evidenced grossly by bluish discoloration 1 to 3 cm in diameter at the injection site of toxin and microscopically by hemorrhage, thrombosis of venules and necrosis of cells. As a control against the factor of trauma from injection, a similar amount (0.25 cc) of physiologic saline was injected into another area of the common duct or gallbladder with no resulting evidence of reaction.

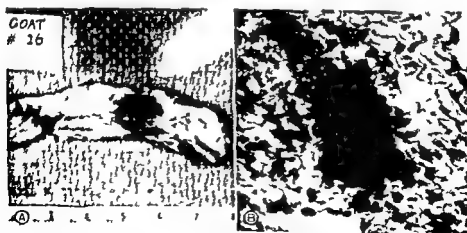


Fig. 1. A: Gross specimen of a common duct from a goat (No. 16) in Group 1. The pointer is directed to the injection site of toxin in which a sharply circumscribed reaction is seen. B: Photomicrograph (700x) of injection site of toxin into wall of gallbladder from a goat (No. 12) in Group 1. This section shows thrombosis of a small vessel and some perivascular cuffing of polymorphonuclear leukocytes.

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STAGE ONE (SENSITIZATION)	
Route of Injection	Application of Methods
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(2) Application to surface of tissue 1 cc	Instillation into lumen of common duct
(3) Into vascular supply of organ 1 cc	Into cystic artery
INCUBATION PERIOD	
24 hr	
STAGE TWO (CHALLENGING)	
Intravenous Injection 1 cc	Into jugular vein

†Modified from Rosenberg²

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ney⁴ demonstrated that toxin readily diffuses through an apparently intact pancreatic ductal system in rabbits and goats. The accidental trauma to the common duct at the time of cholecystectomy might sensitize the duct wall if bacteria were present at that time. Once the common duct is sensitized the circulation of products of gram-negative microorganisms in the blood stream within the proper time interval would elicit the Schwartzman reaction.

SUMMARY

From the data reported herein it would appear that the Schwartzman reaction can be elicited in the biliary tract and may occasionally be a factor in the pathogenesis of obliterative cholangitis. The sensitization process might appear more likely to be produced with the aid of severe trauma (accidental section) to the common duct but possibly could be secondary to the very minor trauma incident to the average operation. This process could explain the unusual progressive reaction in certain cases. Conceivably, it could also be a factor in the development of cholecystitis and hepatitis.

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COMPARATIVE FAT AND FATTY ACID STUDIES IN INTESTINAL MALABSORPTION STATES*

JAMES R. MALM, KEITH REEMTSMA AND HAROLD G. BARKER

The evaluation of malabsorptive states in medical and surgical patients has long presented difficult diagnostic problems. In recent years, following the original method as described by Stanley and Thannhauser¹, a number of investigators have used radioactive iodinated fat to study absorptive patterns in normal and abnormal states. The test as described demonstrated fat malabsorption but gave no clue as to the defect involved.

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Group 2 Instillation Into Lumen of Common Duct Three goats are represented in this group in which the toxin was instilled (sensitization) through a polyethylene tube introduced into that portion of the distal common duct which was defunctionalized. Twenty-four hours later an intravenous (challenging) injection of toxin was given, resulting in a positive reaction in 1 animal evidenced grossly by numerous petechial hemorrhages with an area of confluence 0.5 cm in diameter. Microscopically there was hemorrhage, thrombosis of venules and necrosis of cells.

Group 3 Injection Into Cystic Artery In this group toxin was injected (sensitization) into the cystic artery of three goats followed by an intravenous (challenging) injection of toxin 24 hr later. There was an equivocal reaction in 1 animal evidenced grossly by a darker blue black color of the entire gallbladder. There were also several areas of bluish discoloration of adjacent liver and examination of the cystic artery revealed several small branches to the involved liver. Microscopically the wall of the gallbladder was thicker, because of edema. No areas of hemorrhagic necrosis were seen; however, thrombosis of several small venules was noted.

DISCUSSION

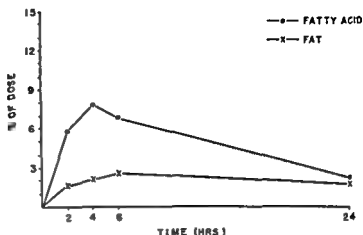
A considerable number of goats (9 of 15) died within 2 to 24 hr following the intravenous challenging injection. This lethal effect of *Shwartzman's* filtrates was noted in rabbits by *Shwartzman*.² He also observed that the concentration of lethal factors somewhat parallels the concentration of toxin in filtrates derived from microorganisms capable of producing toxin in high concentration (both of which are high from meningococcus).³ Since over one half the animals died within 24 hr the conclusion might be drawn that an adequate amount of toxin was given, the reason for negative results was apparently due to the factor termed by *Shwartzman*² as individual susceptibility, and not to an insufficient amount of toxin.

In view of the frequent presence of *E. coli* in the bile of patients with stricture of the common duct, toxin was prepared from an *E. coli* obtained from the bile of patients undergoing choledochoplasty for stricture of the common duct. However, the toxin derived from these strains proved to be less potent than that obtained from the meningococcus. It has been amply demonstrated that toxin derived from other gram-negative microorganisms including some strains of *E. coli* have almost equal ability to elicit the *Shwartzman* reaction.^{3,4}

The clinical implications of these experiments are related to the possibility that the destructive process in the wall of the common duct may, in fact, be due to sensitization by toxins from bacteria present at the time of operation. *Shwartzman*² noted that the reaction following the challenging injection is not localized to the injection site of toxin. The spreading of the area of sensitization is believed to be via the lymphatics of which there is a rich network in the submucosa of the common duct.^{5,7}

Since it has been shown that the disappearance of the toxin from the blood stream is correlated to its appearance in liver extracts (also extracts of spleen),⁷ the bacterial toxins under certain circumstances may subsequently sensitize the common duct either (1) by way of the lymphatic network from the liver or (2) in the bile excreted by the liver. *Thal* and *Brick*

Fig. 2 Percentage of ingested I^{131} dose in circulation at various time intervals in patients with pancreatic insufficiency



and the serum concentrations are about one half as high. The difference is statistically significant and establishes a basis for evaluating the abnormal. The reasons for this difference are not clear and it may be both a function of absorption and disposal from the blood stream. Recent experimental work suggests that oleic acid forms a calcium-oleate phosphate complex in the intestinal lumen that inhibits up to 30 per cent of absorption.⁴ Our data confirm the relatively high fecal loss of I^{131} tagged oleic acid in normals (14.5 per cent to 6.2 per cent of the ingested dose).

A group of patients with pancreatic insufficiency have been studied utilizing the comparative absorption tests. Figure 2 shows the mean serum values obtained in 6 proven cases. It is evident that while there is a marked decrease in fat absorption as compared to the normal curve, oleic acid is absorbed in a normal fashion. This pattern is diagnostic of pancreatic insufficiency and has not been encountered by us in any other disease state in about 200 tests thus far performed.

Figure 3 shows the mean serum values obtained in a group of 14 patients with documented primary intestinal malabsorption. This group included cases of extensive small bowel resection, sprue, Whipple's disease, regional enteritis, and fibrocystic disease. The pattern obtained shows a marked impairment of fat and fatty acid absorption. A similar pattern has been obtained in the presence of pyloric obstruction and in patients with very rapid intestinal transit times. In the absence of these factors the pattern is

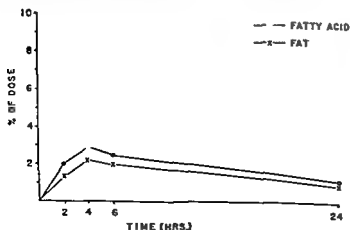


Fig. 3 Percentage of ingested I^{131} dose in circulation at various time intervals in patients with primary intestinal malabsorption

The present test separates the digestive phase of fat utilization from the absorptive phase. Prior to entering the blood stream long chain fats require the action of pancreatic enzymes to hydrolyse the fat molecules, at least in part into constituent fatty acids. The products of this hydrolysis are then transported by a function of the intestinal wall to the lymphatics and capillaries. In the absence of pancreatic enzymes little or no fat absorption can be anticipated but absorption of free fatty acids which remains a function of the intestinal wall independent of pancreatic enzyme should proceed normally. In the presence of primary intestinal dysfunction impaired absorption would be predicted for both fat and its free fatty acid.

METHOD

We have administered I^{131} tagged olive oil and its principal fatty acid oleic acid prepared in our laboratory by a method previously described. The resultant material contains less than 50 microcuries of I^{131} per test dose (9 to 15 cc). The tests are performed at least 48 hr apart and patients are prepared with Lugol's solution for 48 hr prior to testing to suppress uptake of iodine by the thyroid gland. Test meals have been given to fasting patients and a modified breakfast allowed 1 hr after the test has begun. Blood samples were drawn at 2, 4, 6, 8, 12 and 24 hr. Urine collections were made over a 48 hr period and in some cases 72 hr stool collections were made. Serum was separated for analysis and all samples were counted in a well type scintillation counter results being expressed in percentage of the total dose given. Serum samples were expressed as a percentage of the total dose which is in the entire blood volume at the time of sampling based on an assumed serum volume of 15 per cent body weight. Urine and stool data were each expressed in cumulative figures calculated into per cent of dose administered.

RESULTS AND DISCUSSION

The results obtained in normal patients following ingestion of I^{131} tagged olive oil and oleic acid are shown in Figure 1. Our results in normal patients following ingestion of tagged olive oil agree with results obtained by others utilizing commercially prepared I^{131} tagged triolein.³ The serum curve obtained following I^{131} tagged oleic acid is of particular interest as the radioactivity in the serum reaches a peak 2 hr later than that following olive oil.

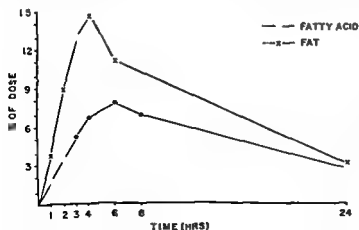


Fig 1 Percentage of ingested I^{131} dose in circulation at various time intervals in normal individuals. Zero time is time of ingestion.

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THIRY FISTULA STUDIES IN STRANGULATION OBSTRUCTION*

GEORGE J WERNITTE AND ISIDORE COHN JR

The advantages of a Thiry fistula preparation for the study of specialized phases of strangulation obstruction have been demonstrated and it has been shown that many of the variables usually encountered in strangulation obstruction can be eliminated^{1, 2}

These experiments were designed to study the role of one of the factors in strangulation obstruction as evaluated under the controlled conditions of the Thiry fistula preparation. The role of specific bacteria was studied in a strangulated segment of bowel without interfering with normal gastrointestinal function causing obstruction or distention or producing the other factors which usually accompany strangulation obstruction. It was hoped that further evaluation of the role of *Cl welchii* in strangulation obstruction could be obtained from these studies.

METHOD

Dogs were operated upon under aseptic conditions and a 50 to 75 cm Thiry fistula established. The omentum was removed as completely as possible to prevent it from forming too many adhesions or acting as a source of vascularization following the second operation. The animals were allowed 2 to 16 months to return to normal. This provided time for the fistula to expel all of its contents except those secreted by the fistula itself. Since there was no continuing source of re introduction of bacteria there was also time for the bacterial content of the fistula to be reduced.

At the second operation a 30 cm central segment of the fistula was deprived of its venous supply by dividing and ligating all the veins to this segment and by dividing and ligating the arteries and veins parallel to the segment at both of its ends. This provided the strangulated isolated open loop of small bowel in which studies could be conducted. A small plastic tube was threaded into the fistula to permit instillation of bacterial cultures deep into the lumen of the fistula.

Rubber tubes were placed in the peritoneal cavity for postoperative aspiration of peritoneal fluid. At frequent intervals specific gravity and white cell counts were determined for peritoneal fluid and blood and the peritoneal fluid was cultured. The animals were maintained on intravenous

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Table I. Cumulative Urinary Recoveries as Per Cent of Dose

SUBSTANCE GIVEN	NORMALS		PANCREATICS		INTESTINALS	
	24 HR	48 HR	24 HR	48 HR	24 HR	48 HR
Fat	70	75	20	40	26	31
Fatty Acid	54	72	38		32	43

diagnostic of malabsorption of the primary intestinal type. It is apparent that pancreatic function can not be evaluated in the presence of intestinal malabsorption by this test.

The study of serum values has proved the most informative means of following the rate of absorption but some valuable quantitative data can be obtained from an analysis of the I^{131} recovered in the urine. Table I shows the comparative cumulative urinary recovery of I^{131} following ingestion of tagged oil and oleic acid. It is apparent that the total urinary excretion of I^{131} is low when malabsorption is present. There seemed to be a wide variation in individual cases which may represent a difference in the rate of absorption not reflected in the serum values. The urine excretion does not appear to be as sensitive a guide as the serum values in most cases and should not be substituted for blood studies. The recovery of I^{131} in the stool correlated well with the anticipated amount of undigested fat based on total dose minus cumulative urine excretion. Stool studies have not been a routine part of the test but were done only to complete the balance studies in selected cases.

A further use for these tests has been in the evaluation of effects of certain medications on fat absorption such as cortisone and pancreatin. The success of pancreatic drainage procedures can be evaluated by repeated studies. The problem of post gastrectomy malabsorption syndrome is being studied at this time. In addition the tests offer a valuable method for further investigation into the mechanism of fat absorption and utilization.

SUMMARY

A comparative absorption test utilizing I^{131} tagged olive oil and oleic acid has been outlined. The absorptive patterns in normal patients are reported and compared to that obtained in pancreatic insufficiency and primary intestinal malabsorption. A low fat and normal fatty acid absorption is consistently found in pancreatic insufficiency while both absorptive curves are low in primary intestinal malabsorption. Cumulative urinary recovery of I^{131} is a quantitative guide to the degree of malabsorption but has considerable individual variation. Some additional uses of the test are discussed.

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In general the white cell counts of the peritoneal fluid and blood showed a gradual rise throughout the important first 18 hr. This is in line with previous observations that the count will continue to rise in an animal that is successfully combatting the condition in contrast to its drop in those animals that are succumbing to the procedure. The animal that died most rapidly had a distinct drop in the blood white cell count but the peritoneal fluid count continued to rise throughout the experiment.

Blood drainage from the fistula occurred at some time or other in each of these experiments in contrast to its usual absence in the previous Thiry fistula experiments.² It was first observed from 2 to 11 hr after strangulation and had usually stopped by 18 hr.

The amount of *Cl. welchii* culture injected was far less than that which had been shown to be without effect in the presence of a normal gastrointestinal mucosa but well above the lethal quantity when injected into the peritoneal cavity in the presence of some suitable culture medium. The rapid expulsion of much of the injected culture and the integrity of the bowel wall acted together to prevent an overdosage of culture in the peritoneal cavity at any one time. The presence of *Cl. welchii* in the early peritoneal fluid in some experiments was attributed to the technique of injecting the culture into the fistula immediately after strangulation and before the peritoneal cavity was closed. In subsequent experiments when cultures were not injected until after the abdomen was closed the early cultures were not positive for *Cl. welchii*.

Seven dogs were subjected to these experiments. Four dogs survived to be reexplored at intervals of 3 to 4 wks at which time the strangulated portion of the fistula was grossly normal. Three dogs died at 34½, 138 and 228 hr (9½ days).

Autopsy on the 2 animals that died at 34½ and 138 hr revealed hemorrhage, gangrene and necrosis in the strangulated segment but no perforations. The strangulated segment was thicker than normal bowel due to hemorrhage in the bowel wall. Necrosis was most marked in the mucosa. The animal that died at 9½ days was the only animal that refused to eat following strangulation. Autopsy showed no changes in the strangulated fistula and death in this experiment was attributed more to inanition than to the experimental procedure. Since the time interval was so long this death was not considered to be related to the experimental procedure and this animal was considered to have survived the strangulation procedure.

DISCUSSION

These studies confirm previous observations about the value of the Thiry fistula preparation for the study of certain phases of strangulation obstruction.^{1, 2} The ease with which these animals can be handled following strangulation is in marked contrast to the difficulty in animals with the usual strangulation obstruction.^{3, 4, 7} The ability of these animals to take food and liquids shortly after strangulation and the absence of excessive vomiting greatly simplifies the fluid and electrolyte balance problems and makes easier the study of other phases of this complex problem. Blood loss still occurs into the wall and lumen of the fistula but neither of these seems to be as pronounced as in the usual strangulation obstruction experiments and this phase of the postoperative care is simplified also.

fluids for 21 to 18 hr after operation. After this fluids and then food were allowed. The dogs were kept in metabolism cages for intake-output studies.

During the first 48 hr after operation a total of 10 ml to 200 ml of *Cl welchii* culture containing 3×10^8 organisms per ml were injected in divided doses into the fistula in an attempt to provide a continuing high concentration of these organisms in the devascularized segment of bowel. Peristalsis of the loop removed a large portion of the injected material so that the amount remaining was far less than that injected. The amount injected was selected after studies showed that dogs with a normal gastrointestinal tract could swallow 300 to 600 ml of such cultures without effect.

For the 1 or 2 days immediately preceding the second operation the fistula was irrigated with antibiotic cultures of *Cl welchii* or both in an attempt to eliminate all other bacteria from the loop following strangulation.

Cultures were obtained from the fistula by injecting and then aspirating saline through a catheter threaded into the fistula. Cultures were obtained at intervals between the 2 operations before any antibiotics or cultures were instilled into the fistula, and at the time of the second operation.

RESULTS

Bacteriologic studies of the lumen of the fistula indicated that sterilization of the fistula did not occur regardless of the length of time the fistula was allowed to drain. The concentration of bacteria undoubtedly decreased but there was not complete removal of bacteria. Temporary sterilization was obtained by irrigation of the fistula with antibiotics but this lasted for only 21 to 18 hr after which the previous bacterial flora returned. Repeated irrigations with large quantities of sterile saline also failed to remove the bacteria from the fistula.

Bacteriologic examination of the peritoneal fluid after strangulation showed that the 1 organisms most commonly found in the fistula—clostridia, coliforms, streptococci, and staphylococci—were found at one time or another in the peritoneal fluid of each animal though they might not all be there at one time. No differences can be detected between the peritoneal fluid bacteriology of the animals that survived and those that died.

Following strangulation the change in color of the strangulated segment was just as dramatic as in past experiments.^{1,2} There was immediate cyanosis and hemorrhage into the bowel wall and mesentery. After recovery from anesthesia these animals returned to a relatively normal status much more quickly than did animals with strangulation obstruction of normal bowel. This was attributed to the lack of vomiting and distention and the absence of obstruction. These animals would eat or drink as soon as water or food was offered. Vomiting was infrequent, occurring only once in 2 experiments and 2 and 3 times in 2 others. It is obvious that fluid and electrolyte loss through vomiting is not a major problem with this type of preparation and thus eliminates one of the major difficulties in the therapy of animals with experimental strangulation obstruction.

The peritoneal fluid aspirated from these animals was usually of a pink color, coagulated on standing, and generally had no odor. Hemolysis was absent or only of minor extent which is in contrast to previous experiments.⁷

MEASUREMENT OF SEQUESTRATION OF RED CELL MASS IN STRANGULATING INTESTINAL OBSTRUCTION UTILIZING RADIOACTIVE Cr^{51} *

PO MYAT YA JOHN I. PERRY, JR., MAURICE SOE THIFIN AND
OWEN H. WANGENSTEIN

Many workers have measured the amount of blood lost from the general circulation in strangulating intestinal obstructions. Scott and Wingensteen¹ compared the weight of the strangulated intestinal segment and its contents and the weight of the peritoneal fluid with that of a similar length of the normal intestine to measure the blood loss into the strangulated loop and the peritoneal fluid. Foster and Husler² placed the strangulated loop in a thin rubber bag and calculated the blood loss from the gain in weight of the rubber bag and its contents with that of comparable controls. Aird³ determined the plasma volume by the vital red method before strangulation and again when the animal was preterminal. He calculated the blood loss from the difference between these two. It was generally agreed from these experiments that blood loss is the most important lethal factor in long loop strangulating venous obstructions.

The introduction of Cr^{51} labelling of red cells has provided an accurate technique for measurement of red cell mass under various conditions. The following study was undertaken to determine the distribution of red cells in the strangulated segment, the peritoneal fluid and the other viscera in experimental strangulating obstructions.

METHOD

Mongrel dogs weighing 6 to 15 kg were utilized. The red cell volume was calculated at the time of intraligging of the red cells. The closed method of red cell volume determination by Cr^{51} using ascorbic acid as a reducing agent for sodium chromate to block plasma radioactivity from entering fresh red cells as described by Reid⁴ was used throughout the experiment. Heparin was used as an anticoagulant in tagging and drawing of the samples. At the time of injection of the Cr^{51} tagged red cells 0.5 to 1 ml of epinephrine was added to insure thorough mixing of the tagged red cells with the cells of the rest of the body. The femoral vein was exposed by a small incision for drawing blood and reinjection of Cr^{51} tagged blood. The sample taken 15 min after injection was drawn from the *opposite* femoral vein for the calculation of red cell volume. A minimum of 3 days was allowed for the plasma radioactivity to decline to negligible amounts. Animals were fasted for 24 hr before the strangulation of the intestine. Lengths of intestine varying from 1 to 1 ft were chosen for the experiment. To insure uniformity the terminal ileum 1 ft proximal to the ileocecal junction was always taken as the distal point of the closed loop and measurements were made from this point. Binding tape was used to occlude the lumen of the bowel at the ends of the segment chosen for strangulation. A window was first made in the mesentery to the bowel at the ends of the

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The failure to achieve sterilization of the fistula in these long term experiments should settle the question of whether a loop removed from the continuity of the gastrointestinal tract can or cannot sterilize itself

The survival of the majority of animals in this series would indicate that venous strangulation of a segment of bowel out of continuity with the remainder of the gastrointestinal tract is not uniformly fatal This is another step in eliminating degenerating tissue alone as a possible source of some toxic substance in strangulation obstruction Even when the segment is irrigated with pure cultures of *Cl welchii* the result is not uniformly fatal The differences between the animals that survived and those that died is not apparent and has suggested a future course of investigation

Harper and Blain⁶ studied the toxemia of intestinal obstruction by utilizing isolated obstructed loops They correlated the toxemia with the presence of distention and bacteria in these loops Their conclusions that an abundant bacterial flora was compatible with life in the absence of distention and that distention was a prerequisite to infection of the intestinal wall by the normal intestinal flora would appear to be confirmed by these experiments which have the added factor of vascular damage to the bowel wall Distention did not occur in these experiments because of the rapid discharge of any fluid or gas formed in the Thiry fistula The additional role that might be played by distention of the Thiry fistula remains to be investigated

The important role of bacteria in strangulation obstruction has been shown by studies which utilized antibiotics to preserve viability in strangulated obstructed bowel^{3, 4} Survival in the current experiments may be due to the absence of some other factors which are present in strangulation obstruction These studies are continuing in an attempt to determine what other factors are necessary to achieve a lethal result

SUMMARY

1 The Thiry fistula technique has been applied to another phase of the problem of strangulation obstruction

2 Venous strangulation of a 30 cm segment of a Thiry fistula is not necessarily fatal even when the fistula has been irrigated with a pure culture of *Cl welchii*

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Table 1 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulations

NO. OF DOGS	LENGTH OF THE LOOP STRANGULATED	PER CENT OF TOTAL INTAKE		SURVIVAL		SEQUESTERED RBC IN STRANGULATED BOWEL AND PERITONEAL FLUID	
		MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
4	1 ft	9%	8-10	19 hr	15-23 hr	3%	20-19
3	2 ft	21%	20-22	11 hr	8-11 hr	11%	33-16%
1	3 ft	31%		6 hr		12%	
2	4 ft	37%	31-42	9 hr	8-10 hr	61%	57-66%

Table 2 Distribution of Cr⁵¹ Labelled Red Blood Cells in Animals Dying of Closed Loop Venous Strangulations

DOX	LENGTH OF STRANGULATED LOOP IN FEET	RED CELLS IN ABDOMINAL CAVITY	RED CELLS IN STRANGULATED LOOP & PERITONEAL FLUID		% RED CELLS IN REMAINDER OF THE BODY
68*	0	36%	0		64%
41*	0	30%	0		70%
40	1	19%	20%		61%
57	1	21%	31%		48%
15	1	20%	10%		40%
54	2	14%	43%		41%
10	3	31%	12%		24%
37	4	27%	57%		16%
58	4	17%	66%		17%

Control Dog, Killed by Electrocutation

* Control Dog Killed with Pentobarbital

general circulation. With the longer loops as much as 61 per cent of the red cells is lost into the strangulated loops and peritoneal cavity. This leaves little room to doubt the importance of blood loss as a major cause of death in closed loop venous strangulating intestinal obstructions.

The distribution of red cells in the viscera, the strangulated loop and the peritoneal fluid of these animals are shown in Table 2 as compared with two control dogs. It could be seen that in animals dying of venous strangulations of the intestine there is no abnormal pooling of red cells in the viscera to account for the production of symptoms of shock and death.

The results with the neomycin group are arranged in Table 3. There was correlation of decreased loss of blood with increased survival time in dogs with the shorter loops. With the longer loops survival time was as short and the total sequestered red cells was essentially the same as in the previous group.

DISCUSSION

This work confirms the work of previous authors that death in venous strangulating intestinal obstructions can occur rapidly from the simple

loop selected. The vessels running adjacent and parallel to the bowel were cut and ligated. The binding tape was passed through this window around the wall of the bowel and tied. One foot of intestine proximal to the loop was measured off and marked with seromuscular stitches of silk and was used as a measure for the whole length of the intestine at the autopsy. The venous supply of the loop was dissected out and cut between ligatures. Animals were allowed to die without any general supportive measures. A sample of blood was drawn before the death of the animal from which was calculated the red cell mass in the abdominal viscera and lungs at the time of death. Autopsy was performed immediately after the death of the animal. The strangulated loop, the normal intestine, abdominal viscera (kidneys, liver, stomach, spleen, colon) and the lungs were taken out after ligating the blood supply of each to prevent spillage of blood. Peritoneal fluid was first taken out with a large syringe; the peritoneal cavity was then washed several times with normal saline and the washings added to the peritoneal fluid. This procedure was done after the strangulated segment was taken out and before the removal of the abdominal viscera. Each organ was placed in a separate beaker, cut into smaller pieces, treated with 10 per cent NaOH solution overnight. Each viscous was homogenized by mixing in a Wiring Blender and by heating in a water bath. Aliquot samples were then obtained for counting in a well type scintillation counter. The red cell mass in the strangulated loop and the peritoneal fluid was calculated from the sample of the blood taken at the time of strangulation. The red cell mass in the viscera was calculated from the sample of the blood taken before the death of the animal.

Two groups of dogs were studied. The first group consisted of 10 animals with closed loop venous strangulations as described above. In the second group of 9 dogs, 15 gm. of neomycin was instilled into the loop for each foot of bowel strangulated. Three dogs in Group 1 died before the blood sample could be drawn and the red cell mass determination for the abdominal viscera and lungs were omitted in these 3.

RESULTS

Observations at autopsy were essentially the same as described by previous workers. The strangulated loop was of dark mahogany color, thickened and distended with thick, foul smelling bloody material. It was found to be ruptured in 3 out of 1 dogs with 1 ft. loops in Group 1. The peritoneum contained from a few ml. to about 250 ml. of thin, dark, foul smelling serosanguinous fluid. The peritoneal fluid was increased in cases of shorter loop strangulations with longer survival time.

In the animals with neomycin instilled into the loop, the foul smell so characteristic of the former group was absent from the strangulated gut contents and the peritoneal fluid. In 2 out of 3 dogs with 1 ft. loops in the neomycin group, there was general peritonitis from the rupture of the proximal nonstrangulated bowel and death may have been due to generalized peritonitis.

The red cell mass sequestered in the strangulated segment and peritoneal fluid in the first group is shown in Table 1. It was found that even though the survival time was longest with the shorter loops, still there was a considerable percentage of the total red cells (mean 35 per cent) lost from the

Table 1 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulations

NO. OF DOGS	LENGTH OF THE LOOP STRANGULATED	PERCENT OF TOTAL INTACT RBC IN STRANGULATED		SURVIVAL		SEQUESTERED RBC IN STRANGULATED BOWEL AND PERITONEAL FLUID	
		MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
4	1 ft	9	8-10	10 hr	13-23 hr	5	20-19
3	2 ft	21	20-22	11 hr	8-14 hr	11 ^a	33-16
1	3 ft	31 ^a		6 hr		12 ^a	
2	4 ft	37	31-42	1 hr	8-10 hr	61	17-66 ^c

Table 2 Distribution of C⁵¹ Labelled Red Blood Cells in Animals Dying of Closed Loop Venous Strangulations

DOG	LENGTH OF STRANGULATED LOOP IN FEET	RED CELLS IN ABDOMINAL VISCERATA	RED CELLS IN STRANGULATED LOOP & PERITONEAL FLUID		RED CELLS IN REMAINDER OF THE BODY
			TOOK A	PERITONEAL FLUID	
68	0	36 ^a	0		64 ^a _b
41**	0	30 _c	0		70 ^a _c
40	1	19 ^a _c	20 ^a _c		61 ^a _b
57	1	21 ^a _b	31 ^a		48 ^a _b
13	1	20 ^a _c	10 ^a _c		10 ^a _c
54	2	11 ^a	1 ^a		41 ^a _b
10	3	31 ^a _b	12 ^a		21 ^a _c
37	1	27	57 ^a		16 ^a _b
58	1	17 ^a _b	66 ^a		17 ^a _b

* Control Dogs killed by Electrocutation
 Control Dogs killed with Fenitobarbital

general circulation. With the longer loops as much as 61 per cent of the red cells is lost into the strangulated loops and peritoneal cavity. This leaves little room to doubt the importance of blood loss as a major cause of death in closed loop venous strangulating intestinal obstructions.

The distribution of red cells in the viscera, the strangulated loop and the peritoneal fluid of these animals are shown in Table 2 as compared with two control dogs. It could be seen that in animals dying of venous strangulations of the intestine there is no abnormal pooling of red cells in the viscera to account for the production of symptoms of shock and death.

The results with the neomycin group are arranged in Table 3. There was correlation of decreased loss of blood with increased survival time in dogs with the shorter loops. With the longer loops survival time was as short and the total sequestered red cells was essentially the same as in the previous group.

DISCUSSION

This work confirms the work of previous authors that death in venous strangulating intestinal obstructions can occur rapidly from the simple

Table 3 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulation of the Intestine (Neomycin Instilled into the Loop)

NO. OF DOGS	LENGTH OF THE LOOP STRAN- GULATED	% OF TOTAL INTESTINE STRANGULATED		SURVIVAL		SEQUESTERED RED CELLS IN STRANGULATED BOWEL AND PERITONEAL FLUID	
		MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
3	1 ft	10%	10-11%	110 hr	84-148 hr	20%	10-33%
3	2 ft	23%	18-29%	32 hr	9-50 hr	46%	42-51%
1	3 ft	27%		7 hr		58%	
2	4 ft	33%	30-36%	8 hr	8-9 hr	63%	53-75%

cause of blood loss alone. Even with moderately short loops comprising about 10 per cent of the total length as much as 49 per cent of the total red cell mass of the body could be lost into the strangulated loop from the circulation. Our results show even greater losses of red cells in short loops than had been reported by other workers.^{1, 2, 3}

In the series of animals treated by instillation of neomycin into the loop the longer survival time occurs with decreased loss of red cells into the loop. This decreased tendency to bleed into the loop has been noted by others in dealing with the open loop venous strangulations.^{6, 7} It raises the question again as to whether decreased blood loss into the loop may not be a significant factor in prolonging the survival in animals with experimental venous strangulations in which neomycin has been instilled into the loop. These experiments in no way detract from the observation of others suggesting suppression of bacterial factor in the prolongation of survival of the animals with venous strangulating obstructions by antibiotics.^{6, 7, 8, 10}

Histologic examination of sections from those with and without neomycin in the strangulated loop at death offered no explanation for the decreased bleeding in the neomycin group although others have described a preservation of near normal histologic appearance of the bowel wall in the open loops with venous strangulation protected by antibiotics.^{6, 7}

SUMMARY

The distribution of Cr⁵¹ labelled red blood cells in animals dying of closed loop venous obstruction of intestine of varying lengths has been studied. Survival time varies inversely with the length of the strangulated segment and the principal cause of death is loss of blood even with 1 ft loops. From the distribution of the red cell mass in the viscera at the time of death there is no evidence to suggest any pooling of the blood in the viscera. With neomycin instilled into the strangulated loop decreased cell loss occurs concomitantly with increased survival in animals with shorter loops but red cell loss as well as survival is almost the same in the control and neomycin groups with longer loops.

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SURGICAL JAUNDICE EXPERIMENTAL EVIDENCE AGAINST THE REGURGITATION THEORY *

HORACE D RITCHIE JOHN H GRINDLAY AND JESSE L BOLLMAN

More than 150 years have passed since the first ligation of the common bile duct was carried out by Saunders. He observed that bile pigments promptly accumulated in the lymph of the thoracic duct. Since then a great deal of work has been done and much has been written on this phenomenon but opinion is still divided as to its significance.

It has been suggested that the phenomenon may be due to regurgitation of bile from the biliary tree into the liver lymph which would then be carried to the thoracic duct and so to the blood itself and that such is the mechanism of surgical jaundice.

Evidence for this view is based mainly on observations of the bilirubin content of thoracic duct lymph from anesthetized dogs within the first few hours after biliary obstruction. Only acute studies are possible with such preparations and it is necessary first to remove the gallbladder.

We have attempted to simulate more closely actual clinical conditions by producing acute repeated and chronic obstruction of the bile ducts in conscious intact or cholecystectomized dogs. The lymph coming from the liver in these conditions has been examined directly for evidence of regurgitation.

METHOD

Fifty healthy mongrel dogs weighing 8 to 12 kg were used in this study and in these animals liver lymph fistulae were established.¹ The experi-

¹ From the Mayo Foundation and Mayo Clinic. The Mayo Foundation, Rochester, Minn. is a part of the Graduate School of the University of Minnesota.

Table 3 *Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulation of the Intestine (Neomycin Instilled into the Loop)*

NO OF DOGS	LENGTH OF THE LOOP STRANGULATED	% OF TOTAL INTESTINE		SURVIVAL		SEQUESTERED RED CELLS IN STRANGULATED BOWEL AND PERITONEAL FLUID	
		MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
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cause of blood loss alone. Even with moderately short loops comprising about 10 per cent of the total length as much as 49 per cent of the total red cell mass of the body could be lost into the strangulated loop from the circulation. Our results show even greater losses of red cells in short loops than had been reported by other workers.^{1, 2, 3}

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COMMON DUCT PRESSURES FOLLOWING SPHINCTEROTOMY*

W H BROWN T EARLEY AND H EISEMAN

The physiologic rationale for sphincterotomy in the treatment of chronic pancreatitis depends upon the prevention of biliary reflux into the pancreatic duct by decreasing the intraluminal choledochal pressure^{1,2,3}. Although common duct pressures occasionally have been measured immediately after sphincterotomy⁴ there have been no such studies covering a prolonged period following this operation.

This is a report of the emptying pressures in the common duct of dogs over a prolonged period following sphincterotomy and thus is a measurement of the efficacy of this operation in preventing biliary regurgitation into the pancreas.

METHOD

Intraductile free flow pressures have been determined in a series of 18 dogs via a common duct T tube exteriorized through a right upper quadrant stab wound.

Grindlay's technique⁵ for maintaining the catheter in place was found to be excellent for pressure measurements in the immediate postoperative period but the animals in this study invariably extricated the catheters within 2 to 4 wks. Fixation of the T tubes within a windowed and buried plaster cast resulted in respiratory complications in a majority of the dogs so treated. In 18 animals the T tube was fitted intraperitoneally to a plastic cannula which penetrated the abdominal wall and was firmly fixed by an adjustable flange that screwed over the cannula and rested on the skin (Fig. 1).

With the unanesthetized fasting animal lying on his left side physiologic saline was slowly (5 ml/min) injected into the T tube and pressures recorded on a spinal manometer inserted into the system via a glass Y tube. The level of the porta hepatis was taken as the base line. The pressure within the system gradually increased as fluid was injected into the system.

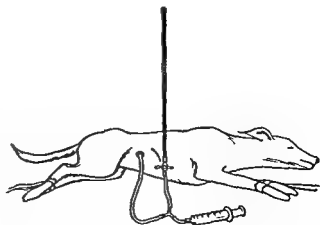


Fig. 1 Diagram of method for determining common duct free flow pressure.

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mon bile duct in the normal dog most of the bilirubin which accumulates in the blood stream has been discharged into it directly from the biliary tree during its passage through the liver

If these findings could be regarded as valid for man they would suggest that regurgitation of bile into the liver lymph during biliary obstruction could occur only if the gallbladder had been removed or was nonfunctioning and that this phase would be over about 24 hr after the obstruction had been established. Its contribution to the ultimate jaundice would therefore be a minor one.

Moreover, should the blockage relieve itself and as in obstruction by calculus recur soon afterward or if a functioning gallbladder were present, as in many neoplastic obstructive conditions there would appear to be little evidence that regurgitation into the lymph could play any part in the production of the ensuing jaundice.

SUMMARY

1 Acute repeated and chronic obstruction of the common bile duct has been produced in 50 conscious dogs. The concentrations of bilirubin in the lymph coming from the liver and in the blood of these animals have been measured at intervals for periods of up to 4 days. The data have been scrutinized for evidence of regurgitation of bilirubin into the liver lymph.

2 Evidence of such regurgitation was found only during the first 24 hr of a first bout of obstruction in the cholecystectomized animals.

3 No evidence of regurgitation was found (a) after the first 24 hr in the cholecystectomized animals (b) during any subsequent biliary obstruction in the cholecystectomized animals (c) in acute obstruction when the gallbladder was present or (d) during chronic obstruction in animals with or without a gallbladder.

4 Sulfobromophthalein studies have confirmed these findings.

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sure of 30 cm. of saline prior to operation. In a number of animals resistance to flow was already perceptible at the time of laparotomy closure. Twenty-four hr. postoperatively the free flow pressures were approximately 30 per cent below the preoperative levels and usually remained depressed for the next 5 days. Thereafter the pressure gradually returned to preoperative levels and remained so for the balance of the experiment. Representative experiments are illustrated in Figure 2. Neither double cut sphincterotomy nor leaving the gallbladder intact altered the basic free flow pressure response.

An increase in free flow pressure following the instillation of hydrochloric acid was noted in each case prior to sphincterotomy, a pattern which disappeared only for 24 to 48 hr. following operation. Thereafter acid stimulation produced a progressively greater response which within a week resulted in a resistance to flow equal to that found preoperatively.

Injection of saline into the T tube at the time the animals were sacrificed 6 to 8 wks. following sphincterotomy produced a thin high trajectory stream from the common duct into the duodenum similar to that found prior to sphincterotomy, and in contrast to the wide diameter gush noted immediately following sphincter section.

Microscopic examination of the sphincters revealed fibrous union between the musculature surrounding the duct at the site of its previous section regardless of the type of sphincterotomy previously performed.

DISCUSSION

These experiments indicate that sphincterotomy diminished the choledochal free flow pressure in the dog for only a few days following operation. They further indicate that the rise in emptying pressure resulting from the injection of hydrochloric acid into the duodenum that is said^{3, 6} to be indicative of sphincter action persists to a significant though diminished degree following section of the sphincter and returns to preoperative levels within a week. They further suggest that choledochal free flow pressures are not primarily regulated by sphincter action or if so governed are not permanently affected by sphincterotomy.

Factors other than sphincter of Oddi contraction that might alter choledochal emptying pressure include the pinchcock action of the duodenal wall as the duct passes through it obliquely or the contractile action of the circular smooth muscle fibers in the wall of the common duct proximal to its entrance into the duodenum. Duodenal pressures are not transmitted directly to the common duct.⁶

The immediate rise in free flow pressure after operation may be due to wound edema at the site of sphincterotomy but this would be expected to subside within a few days. Healing of the singly or doubly sectioned sphincter on the other hand may account for the late return of sphincter tone as the encircling muscle fibers are joined by fibrous scar.

Whatever the physiologic explanation may be sphincterotomy as here performed does not permanently affect the free flow pressures of the common bile duct in the dog nor does it permanently alter the response of such pressure to hydrochloric acid instillation into the duodenum. These experiments therefore throw serious doubt on the physiologic rationale of sphincterotomy in management of chronic pancreatitis.

and then fell suddenly at the moment it emptied into the duodenum. The pressure required to overcome the resistance to flow has been called the free flow pressure. The mean of 10 consecutive measurements performed at 1 to 3 min intervals has been used for each experiment, and has varied routinely less than 5 cm of saline. Control free flow pressures were recorded weekly for 3 wks after insertion of the T tube and cholecystectomy. Pressures were then recorded immediately prior to sphincterotomy at the time of opening the duodenum after sphincter section immediately following duodenal closure at the time of laparotomy closure daily for 7 days and finally at weekly intervals 6 times thereafter. Following each such pressure measurement 8 ml of 1/N HCl was injected into the duodenum via the T tube and free flow pressures measured immediately thereafter.

Extreme care was taken in the performance of the transduodenal sphincterotomy to cut the intramural muscle fibers as well as those of the sphincter and to assure that an uninterrupted stream flowed into the duodenum. This necessitated division of the impuller and proximal section of the sphincter fibers for approximately 2.0 cm.

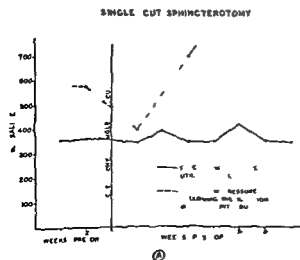
In 6 animals the sphincter of Oddi was sectioned in 2 places at 90° angles to each other (6 and 3 o'clock) in an effort to produce biliary incontinence.

At the end of 8 wks all animals were sacrificed and the common duct and sphincter of Oddi examined grossly and microscopically to assure complete severance of all sphincter fibers and to find evidence of healing.

RESULTS

Immediately following sphincterotomy the patulent sphincter allowed a free flow from the common duct into the duodenum and there was but an imperceptible resistance to injection compared to the mean free flow pres-

Fig 2 Representative experiments of common duct free flow pressures showing response to hydrochloric acid injection before and after sphincterotomy. a) Single cut sphincterotomy. b) Double cut sphincterotomy.



patients who survived for 2 mos or more after operation have been selected for the present report and certain aspects of sodium metabolism as it relates to ascites will be stressed

METHOD

Following an initial 3 to 5 day period during which sodium intake was normal and high protein high caloric low sodium diet (10 mEq/day) was instituted. Some patients stabilized their ascites on this program alone others required occasional paracenteses or courses of ion exchange resins, but all showed residual ascites at the time of operation. Low serum albumin levels were corrected by administration of intravenous albumin. All patients were maintained on complete water electrolyte and nitrogen balance for periods of 1 to 8 wks preoperatively and 3 to 8 wks postoperatively. End to side or side to side portacaval shunts were performed following which sodium intake was maintained at a low level as long as urinary sodium output remained low. Thereafter a more liberal sodium intake was occasionally permitted, but intake was limited to an amount less than that being excreted daily in the urine. The usual laboratory tests of liver function were obtained at frequent intervals throughout the preoperative and postoperative periods. The data on sodium intake and urinary sodium output originally determined and calculated on a daily basis are presented here as averages over weekly periods.

RESULTS

The 7 patients presented fall into 2 distinct groups. Four (Group 2) have survived 2 to 6 yrs after portacaval shunts, and none has shown reaccumulation of ascites. The remaining 3 patients (Group 1) died 2, 3, and 18 mos after operation and all showed recurrent ascites. As far as could be determined preoperatively the 2 groups were comparable in severity of liver damage and clinical condition.

Serum Sodium levels. The mean values of the preoperative serum sodium levels were essentially the same in both groups: 134.2 and 135.5 mEq/L in Groups 1 and 2 respectively. However, Group 1 patients showed a rise in the postoperative serum sodium concentration to a mean value of 138.6 mEq, whereas Group 2 patients showed a decreasing serum sodium level with a mean postoperative value of 129.2 mEq.

Urinary Sodium excretion. The mean values of the preoperative urinary sodium values were approximately the same in both groups: 2.3 mEq/day in Group 1 and 2.7 mEq/day in Group 2. There was a marked difference, however, in the postoperative period between the 2 groups. Urinary sodium excretion for Group 2 patients, who subsequently reaccumulated ascites and died, remained at a low level throughout the entire postoperative period, the mean urinary sodium values being 1.9 mEq/day. The Group 1 patients, however, demonstrated a consistent increase in urinary sodium levels in the postoperative period, usually starting by at least the 3rd or 4th wk. The mean daily urinary sodium level was 3.8 mEq during the first postoperative week, 9.7 mEq for the second week, 21.8 mEq for the third week, and 37.4 mEq for the fourth week. Generally no increase in sodium intake above 10 mEq/day was permitted until the urinary output reached 15–20 mEq daily.

SUMMARY

- 1 Free flow pressures within the common duct have been measured in a series of 18 dogs at representative periods before and for prolonged periods following both single and double section of the sphincter of Oddi
- 2 Resistance to biliary emptying into the duodenum is thus measured returns to presphincterotomy level almost immediately following operation and remains elevated for a prolonged period thereafter
- 3 The free flow pressure response to intraduodenal hydrochloric acid is but temporarily reduced following complete section of the sphincter
- 4 It is concluded that sphincterotomy in dogs is not permanently effective in reducing the resistance to flow from the common duct into the duodenum

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CHANGES IN SODIUM METABOLISM FOLLOWING PORTACAVAL SHUNTS IN PATIENTS WITH CIRRHOSIS AND ASCITES*

KEITH REENTSMA ROBERT E CARLSON DAVID V HABIB AND HAROLD G BARKER

Patients with cirrhosis portal hypertension and ascites who undergo portacaval shunt operations frequently show clearing of ascites in the postoperative period. The majority of such patients have minimal ascites which responds quickly to mercurial diuretics and therefore require no special preoperative care. However cirrhotics who have long standing severe ascites which is unresponsive to mercurial diuretics constitute a special group of patients with whom this study is concerned. The 7 most completely studied

*From the Department of Surgery, Columbia University College of Physicians and Surgeons and the Surgical Service, Presbyterian Hospital, New York. Supported in part by U S Public Health Grant RG 2926 (C-1).

5 Failure to start excreting at least 10 mEq of sodium daily by the third postoperative week was a poor prognostic sign

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A COMPARISON OF LIVER FUNCTION FOLLOWING ECK FISTULA AND PORTACAVAL TRANSPOSITION*

WILLIAM SILEN DEAN L MAWDSLEY WILLIAM L WEIRICH HAROLD A HARPER AND H J MCCORRLE

It has been observed that the function of the liver is impaired in dogs in which an Eck fistula has been surgically produced.^{1, 2, 3} The classical syndrome of meat intoxication may easily be induced in dogs with Eck fistulas⁴ but as recently shown in this laboratory,⁵ this symptom complex can not be elicited in animals with surgically produced transposition of the portal vein and vena cava even after prolonged administration of raw meat. A comparative study of the function of the liver before and at several intervals after each of these 2 operations was therefore undertaken in an effort to discover some of the causes for the apparent differences in function that were observed.

METHOD

All surgical procedures were carried out with the use of intravenous sodium nembutal anesthesia and strict aseptic precautions. In 8 dogs (Group 1) an Eck fistula was produced through a right thoracoabdominal incision. Six dogs (Group 2) underwent the operation of transposition of the portal

*From the Surgical Research Laboratories of the University of California School of Medicine San Francisco. Supported by the Christine Breon Fund for Medical Research and the United States Public Health Service Grant No. A 1053.

Liver Function Studies No consistent pattern emerged from analysis of pre and postoperative serum albumin levels cephalin flocculation tests alkaline phosphatase determinations or homsulphonthiazolein excretion values. There were no marked differences between Group 1 and Group 2 patients and no consistent differences in individual patients between preoperative and postoperative periods.

DISCUSSION

This study demonstrates that in a group of patients with cirrhosis and ascites subjected to portacaval shunts the postoperative changes in sodium metabolism are closely correlated with the clinical course of the patient including the disappearance or recurrence of ascites.

The exact mechanisms responsible for the disappearance of ascites following portacaval shunts are obscure. The relationship between extrahepatic portal pressure and ascites formation has not been clearly defined although experimental evidence suggests that they may not be closely correlated. The delayed onset of urinary sodium diuresis for 3 wks or more as we and others¹ have seen in some patients after reduction in portal pressure is not easily explained on the mechanical effect of reduced portal pressure, although sodium retention for perhaps 10 to 14 days might be expected to follow a procedure of the magnitude of a portacaval shunt. One further point against the mechanical theory is the lack of correlation between presence or absence of ascites and the degree of portal hypertension.² A second possible mechanism concerns the role of plasma proteins particularly albumin but this study and others³ suggest that serum albumin levels are not correlated with ascites formation.

During recent years increasing attention has been focused on sodium metabolism in ascites^{3,4} and its relationships to adrenal and pituitary function.^{5,6,7} It is possible that the disappearance of ascites following portacaval shunts may be related to a humoral mechanism influencing sodium distribution and excretion. The liver may play a detoxifying or other role in such a system and it is possible that the relief of portal hypertension may lead to subtle improvement in this particular liver function not detectable by the ordinary tests. That this may be a factor is suggested by the observation that serum sodium levels and urinary sodium excretion are consistently elevated in patients who show no recurrence of ascites following portacaval shunts.

SUMMARY

1 Seven selected patients with cirrhosis portal hypertension and severe ascites are reported. They were studied by balance techniques in the surgical metabolism unit for 4 to 8 wks before and 3 to 8 wks after portacaval shunting.

2 The group of 4 patients who have shown no recurrence of ascites for 2 to 6 yrs following shunts all demonstrated rising serum sodium levels and increasing urinary sodium output in the postoperative period.

3 A group of 3 patients who demonstrated recurrent ascites and subsequently died showed persisting or increasingly pronounced hyponatremia and continued low urinary sodium levels in the postoperative period.

4 Liver function studies and serum albumin levels could not be correlated with the clinical course.

was found to be occluded at autopsy although the other was still patent. As a result this animal died in Eck fistula together with obstruction of the return of venous blood from the kidneys and lower extremities. With respect to liver function, clinical signs and symptoms, and relation of liver weight to body weight the findings in this dog were typically those of an animal with an Eck fistula. Microscopic examination of the kidneys revealed no abnormality.

There was a rather marked difference in the general condition of the 2 groups of dogs as the experiment progressed. All of the animals with Eck fistulas lost weight and had a tendency to anorexia, lassitude, and polydipsia. The mean weight loss of these dogs was 2.34 kg over a 3 mo period. In contrast, most of the dogs with portacaval transposition either maintained a constant weight or gained small amounts of weight. Furthermore, these dogs maintained normal activity and appetite and none developed the syndrome of lassitude, anorexia, and polydipsia observed in the animals with Eck fistulas.

The total circulating plasma albumin decreased in every dog with an Eck fistula, the mean loss being 19.9 gm. On the other hand, the total circulating albumin increased (with one exception) in all animals with portacaval transposition, the mean gain being 13.8 gm over a 3 mo period. The globulin content of the plasma and the results of the thymol turbidity test remained normal in all of the animals in both groups.

Clearance of bromsulfalein decreased in every animal with an Eck fistula but rose markedly in every dog with a portacaval transposition (Fig 2a).

In both groups of dogs tolerance to orally administered ammonium lactate was diminished following operation (Fig 2b). This was much more evident, however, in the dogs with Eck fistulas. The mean values for the peak levels of ammonia nitrogen attained in the tolerance tests were 4.56 $\mu\text{g/ml}$ for the animals in Group 1 and 3.97 $\mu\text{g/ml}$ for those in Group 2, despite the fact that those with Eck fistulas received only one-half the dose of ammonium lactate given to the animals with portacaval transposition.

The mean fasting level of ammonia nitrogen in the blood of all of these dogs increased within 3 mo after operation (Fig 2b). The increase was slight in the animals with portacaval transposition but very marked in those with Eck fistulas. These observations on ammonia tolerance suggest that the response of the 2 groups of experimental animals to the feeding of large quantities of raw meat may be attributed to the differences in the clearance of ammonia that existed in these animals in accordance with the adequacy of the blood supply to the livers.

The gross appearance of the livers was normal in both groups of experimental animals except that the livers of the dogs with an Eck fistula were usually smaller. This finding is similar to that of Whipple¹ who described marked atrophy of the liver in dogs with Eck fistulas. On the other hand, the relation between the weights of the livers and the body weights in the dogs with portacaval transposition compared favorably with the normal.

Histologic studies of the livers of the animals with Eck fistulas revealed a tendency to the accumulation of fat in the central zones of the lobules and a loss of hepatic cells adjacent to the central vein. The glycogen content of the cells was also decreased. These changes were present in the livers of all of the dogs in Group 1, varying only in degree with respect to a given speci-

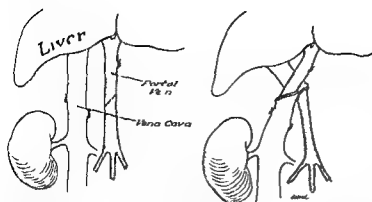


Fig 1 Diagram showing the technique of transposing the portal vein and vena cava

vein and vena cava which was carried out in the manner described by Child⁶ (Fig 1). The dogs were kept on the usual kennel rations throughout the experiment.

In 1 dog with Eck fistula and in 5 dogs with portacaval transpositions studies of liver function were carried out preoperatively and at intervals of 1 wk and 1, 2 and 3 mo after operation. These studies included measurement of total serum protein as well as albumin and globulin, thymol turbidity, clearance of bromsulfalein and tolerance to orally administered ammonium lactate. The clearance of bromsulfalein was determined by the method of Goodman.⁷ By extrapolation of the bromsulfalein concentration to zero time, the plasma volume of each animal was calculated and the plasma volumes so obtained together with the measured serum albumin concentrations were used to compute the amounts of total circulating protein.

Tolerance to orally administered ammonium lactate was determined in the following manner. After a sample of blood had been taken from the fasting animal for estimation of the level of ammonia, a test dose of 0.93 gm ammonium lactate/kg of body weight was given to the animals in Group 2 (portacaval transposition), one half that dose was administered in the same way to the animals with Eck fistula (Group 1) since preliminary studies had shown that these dogs tolerated poorly the larger dose. Specimens of blood were drawn at specified intervals after the test dose and the ammonia content of each sample was promptly determined using a modification of the microdiffusion method of Conway.⁸

The body weight of each of the animals in the fasting state was recorded each time the laboratory tests were performed. At the end of these studies the dogs were sacrificed. At autopsy the livers were removed and weighed immediately in the fresh state and specimens of 1 of the kidneys and of 3 different lobes of the livers were obtained for microscopic examination. In addition to the usual stains special glycogen (Schiff's stain) and fat (Oil Red O) stains were used to study the liver specimens.

RESULTS

All of the dogs survived the test period. The vascular anastomoses between the portal vein and vena cava were found to be patent in all of the animals of both groups with the exception of 1 dog with a portacaval transposition. The repair of a tear in the wall of the portal vein at the time of operation had narrowed the lumen of the anastomosis between the distal vena cava and the proximal portal vein and it was this anastomosis which

was found to be occluded at autopsy although the other was still patent. As a result this animal had an Eck fistula together with obstruction of the return of venous blood from the kidneys and lower extremities. With respect to liver function, clinical signs and symptoms and relation of liver weight to body weight the findings in this dog were typically those of an animal with an Eck fistula. Microscopic examination of the kidneys revealed no abnormality.

There was a rather marked difference in the general condition of the 2 groups of dogs as the experiment progressed. All of the animals with Eck fistulas lost weight and had a tendency to anorexia, lassitude and polydipsia. The mean weight loss of these dogs was 2.34 kg over a 3 mo period. In contrast, most of the dogs with portacaval transposition either maintained a constant weight or gained small amounts of weight. Furthermore, these dogs maintained normal activity and appetite and none developed the syndrome of lassitude, anorexia and polydipsia observed in the animals with Eck fistulas.

The total circulating plasma albumin decreased in every dog with an Eck fistula, the mean loss being 19.9 gm. On the other hand, the total circulating albumin increased (with one exception) in all animals with portacaval transposition, the mean gain being 13.8 gm over a 3 mo period. The globulin content of the plasma and the results of the thymol turbidity test remained normal in all of the animals in both groups.

Clearance of bromsulfalein decreased in every animal with an Eck fistula but rose markedly in every dog with a portacaval transposition (Fig. 2a).

In both groups of dogs, tolerance to orally administered ammonium lactate was diminished following operation (Fig. 2b). This was much more evident, however, in the dogs with Eck fistulas. The mean values for the peak levels of ammonia nitrogen attained in the tolerance tests were 4.56 $\mu\text{g}/\text{ml}$ for the animals in Group 1 and 3.97 $\mu\text{g}/\text{ml}$ for those in Group 2, despite the fact that those with Eck fistulas received only one-half the dose of ammonium lactate given to the animals with portacaval transposition.

The mean fasting level of ammonia nitrogen in the blood of all of these dogs increased within 3 mo after operation (Fig. 2b). The increase was slight in the animals with portacaval transposition but very marked in those with Eck fistulas. These observations on ammonia tolerance suggest that the response of the 2 groups of experimental animals to the feeding of large quantities of raw meat may be attributed to the differences in the clearance of ammonia that existed in these animals in accordance with the adequacy of the blood supply to the livers.

The gross appearance of the livers was normal in both groups of experimental animals except that the livers of the dogs with an Eck fistula were usually smaller. This finding is similar to that of Whipple¹ who described marked atrophy of the liver in dogs with Eck fistulas. On the other hand, the relation between the weights of the livers and the body weights in the dogs with portacaval transposition compared favorably with the normal.

Histologic studies of the livers of the animals with Eck fistulas revealed a tendency to the accumulation of fat in the central zones of the lobules and a loss of hepatic cells adjacent to the central vein. The glycogen content of the cells was also decreased. These changes were present in the livers of all of the dogs in Group 1, varying only in degree with respect to a given speci-

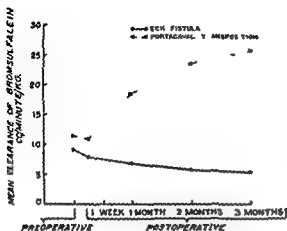
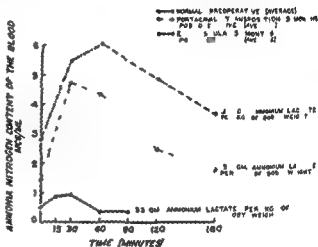


Fig. 1 (a) Graph showing the mean values for bromsulfalein clearance for animals with Eck fistulas and those with portacaval transposition

Fig. 2 (b) Graph showing ammonia tolerance in dogs with Eck fistulas as compared to normal dogs and those with portacaval transposition



men The livers of the dogs with portacaval transposition were entirely normal and glycogen and fat were present in normal amounts

The kidneys of both groups of animals were normal both grossly and microscopically

SUMMARY

A group of dogs with Eck fistulas and another group with transposition of the portal vein and vena cava were prepared to compare the effects of these 2 operations. The animals with Eck fistulas lost weight and their serum albumin levels, bromsulfalein clearance, and tolerance to ammonium lactate were markedly less than those of normal animals or those with portacaval transposition. Histologic examination of the livers of the animals with Eck fistulas revealed fatty infiltration, decrease of glycogen, and loss of cells in the central zone. In contrast, all the findings in the dogs with portacaval transposition were very similar to those of normal animals.

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METABOLIC EFFECTS OF TRANSPOSITION ON THE PORTAL VEIN AND INFERIOR VENA CAVA*

W B SUMMERS, W C MATTIEI AND B JISEMAN

Transposition of the portal vein and inferior vena cava was first performed in dogs by Child¹ and coworkers in 1953 in a study of liver regeneration. They commented on the frequent occurrence of shock that followed the procedure—a complication since noted by others.² This paper is a study of some of the metabolic effects of portacaval transposition with particular emphasis on the cause and avoidance of postoperative shock that so frequently accompanies this operation.

METHOD

Transposition of the portal vein and inferior vena cava has been performed in a series of 61 adult mongrel dogs anesthetized with intravenous sodium pentobarbital anesthesia (35 to 50 mg/kg body weight), and artificially respired via an indwelling endotracheal tube. With the animal in the left lateral position a right thoracoabdominal incision was made and the portal vein mobilized from the hilus of the liver to its emergence from the pancreas. The inferior vena cava was similarly mobilized from its entrance into the right lobe of the liver to the right renal vein.

The veins were transected between vascular clamps, transposed and the anastomosis performed with 5-0 arterial silk (Fig. 1). Since occlusion of the portal system in dogs is poorly tolerated for more than 15 min.,³ the distal portal vein was first anastomosed to the proximal cava.

From the outset these animals tolerated the procedure poorly. The operation itself was usually uneventful, requiring a total of 10 to 70 min. and as

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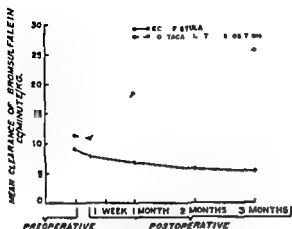
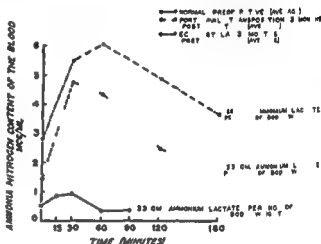


Fig 2 (a) Graph showing the mean values for bromsulfalein clearance for animals with Lck fistulas and those with portacaval transposition

Fig 2 (b) Graph showing ammonia tolerance in dogs with Lck fistulas as compared to normal dogs and those with portacaval transposition



men The livers of the dogs with portacaval transposition were entirely normal and glycogen and fat were present in normal amounts

The kidneys of both groups of animals were normal both grossly and microscopically

SUMMARY

A group of dogs with Lck fistulas and another group with transposition of the portal vein and vena cava were prepared to compare the effects of these 2 operations. The animals with Lck fistulas lost weight and their serum albumin levels, bromsulfalein clearance, and tolerance to ammonium lactate were markedly less than those of normal animals or those with portacaval transposition. Histologic examination of the livers of the animals with Lck fistulas revealed fatty infiltration, decrease of glycogen, and loss of cells in the central zone. In contrast, all the findings in the dogs with portacaval transposition were very similar to those of normal animals.

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Table 1 Summary of Procedures Employed to Avoid Postoperative Shock

PROCEDURE	NO DOGS	DIED FROM KNOWN TECHNICAL CAUSE	DIED IN SHOCK	SURVIVED
A No Supportive Therapy	7	1	3	0
B Diminution of deleterious effect of portal venous occlusion				
1 Blood transfusion	10	5	0	1
2 Hypothermia	2	0	2	0
3 Hypothermia plus clamping thoracic aorta	1	0	1	0
4 Anastomosis over siliconized tube	2	0	2	0
Use of non obstructing anastomosis clamp	2	0	2	0
6 Bypass shunts (2 Methods)	5	1	4	0
7 Splenectomy	3	1	2	0
8 Staged procedure avoiding portal occlusion (2 Methods)	8	0	8	0
C Reversal of order of anastomosis	2	1	1	0
D Oxygenation of liver				
1 Temporary splenic artery portal vein shunt	3	0	3	0
2 Permanent splenic artery portal vein shunt	2	0	2	0
E Adrenal cortical replacement therapy				
1 Intravenous compound F at operation only	1	0	1	0
2 Pre and postoperative cor- ticone therapy	16	4	0	12
Totals	64	14	37	13

unobstructed upper extremities. In 3 dogs polyethylene tubes were placed in the portal vein and inferior vena cava and blood pumped via a syringe and 3 way stopcock into the jugular vein during the period of venous occlusion. In 2 other temporarily heparinized animals a Sigmamotor pump as currently employed in the pump oxygenator⁴ was substituted for the syringes so that continuous perfusion was attained. Of the 5 animals in this group 1 expired because of a technical cause and the other 4 succumbed in progressive shock 2 to 12 hr after an otherwise uneventful operative procedure.

Performance of the anastomoses in the reverse manner with flow first being resumed from the cava into the liver did not avoid postoperative death from shock in 3 animals.

In 8 animals portal venous interruption was entirely avoided by performing a preliminary side-to-side portocaval anastomosis. In half of these animals a vein graft was interposed end-to-end between the portal vein severed above its anastomosis to the vena cava and to the severed cava inferiorly. In the other 4 animals a vein graft was placed into the side of the intact distal

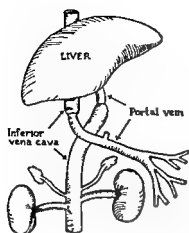


Fig. 1 Diagram of the completed portacaval venous transposition

sociated with minimal blood loss but the animals routinely expired in progressive and uncontrolled shock 2 to 18 hr following operation. Of the 64 animals included in this study 51 animals expired in shock during the immediate postoperative period (Table 1). Of these 14 animals expired from a known technical cause and are not further included in the statistical analysis. In 8 animals this progressive shock was accompanied by a bloody diarrhea appearing in the immediate postoperative period and which was at first assumed to be due to prolonged portal venous occlusion.

The first 7 animals were operated upon without supportive blood transfusions. 4 died of technical difficulties and 3 of unexplained shock 2 to 12 hr following operation.

In the next 10 animals compatible blood transfusions (mean of 270 ml) were utilized to avoid hypotension. All transfusions were given into the upper extremities in order to avoid overloading the lower extremities during temporary venous occlusion. Four animals died because of technical difficulties and 5 others expired in the immediate postoperative period in uncontrollable shock and with bloody diarrhea. The only survival in this group had a previous splenorenal anastomosis.

At this point steps were taken to diminish the time and deleterious effect of portal venous occlusion. Hypothermia (83.5 to 84.5°F) was employed in 3 dogs during the procedure and in 1 animal was combined with cross clamping of the thoracic aorta during the period of portal venous occlusion. Despite these measures all 3 animals died in postoperative shock. Portal occlusion time was reduced to 3 to 5 min and in 2 dogs anastomoses were performed over siliconized polyethylene tubes so that portal flow was interrupted less than 2 min. In 2 other animals vascular occlusion time was further minimized by employment of a vascular clamp of our own design which allows continued blood flow during the period of vascular suture. In all of these cases however the animals died in postoperative shock.

In order to further reduce vascular pooling within the portal bed during venous occlusion splenectomy was performed on 3 dogs prior to performing transposition of the portal vein and inferior cava. One animal died of a technical difficulty but the other 2 expired after operation in uncontrollable shock.

Attempts were then made during the period of venous occlusion to pump blood from below the point of caval occlusion in the lower extremities to the

It seems evident that the shock occurring following portacaval transposition is due to hypoadrenalism since all animals died when not given supportive adrenal cortical replacement therapy and none died from unexplained causes when such replacement was practiced. The combination of the stress of operation plus the hepatic inactivation of the adrenal corticoids proved lethal to the unsupported animal.

Bloody diarrhea is a known clinical manifestation of adrenal cortical insufficiency⁸ and its occurrence in a number of the animals dying in shock might be ascribed in part to this metabolic defect as well as to prolonged portal occlusion.

SUMMARY

The lethal postoperative shock associated with transposition of the portal vein and inferior vena cava above the adrenal vein has been shown to be due to adrenal insufficiency. This results from hepatic inactivation of adrenal corticoids which are diverted directly into the liver following this operation.

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vena cava and anastomosed to the severed portal vein at the hilus of the liver 1 wk following the original side to side portacaval anastomosis. Vena caval flow was then diverted to the liver via the graft by the placement of a ligature above the graft anastomosis. Both of these procedures avoided even temporary occlusion of portal venous drainage and in none of the animals were there technical difficulties yet all 8 animals expired in unexplained postoperative shock.

In the belief that the unexplained postoperative shock might be due to liver damage and to the effect of hepatic anoxia⁶ attempts were made to perfuse oxygenated blood into the liver during and after the operative procedure. In 5 dogs this was attained by anastomoses of the splenic artery into the portal vein close to the hilus of the liver. In 2 animals the anastomosis was left in place after the operation while in 3 other animals the splenic artery was ligated shortly after completion of the venous transposition. Postoperatively oxygenated blood was infused into the liver via the femoral vein but all of the animals expired within 18 hr of unexplained shock.

This experience suggested that a metabolic rather than a technical difficulty was responsible for the shock following portacaval venous transposition since all adrenal venous drainage was diverted directly into the liver following this operation. Since adrenal corticoids are known to be inactivated by the liver^{7,8} functional hypoadrenal corticoidism might thus be produced.

On this basis the animals were prepared preoperatively with 3 daily intramuscular injections of 50 mg of cortisone acetate and maintained on a continuous infusion of 50 mg of Compound F during operation. Postoperatively cortisone 50 mg daily was given for from 2 to 8 days. Of the 16 animals given such adrenal hormone therapy 4 died postoperatively from known technical causes but the remaining 12 dogs were long term survivors and had an uneventful postoperative course without evidence of hypotension.

One animal given Compound F during the operation without preoperative preparation expired in shock shortly after the infusion was discontinued.

DISCUSSION

In the original description of portacaval transposition Child¹ noted the frequent occurrence of postoperative mortality due to shock. Such mortality has been confirmed by others² but has been materially reduced by temporary clamping of the superior mesenteric artery during the period of venous occlusion and by giving blood transfusions during and after the procedure.^{1,2} In the operation as described by Child the right adrenal vein is severed in order to obtain more adequate mobility of the vena cava whereas in this study the adrenal veins were left intact and the anastomosis performed proximal to this point. In the former technique some of the adrenal drainage might therefore bypass the liver whereas in the latter case all adrenal hormones flow directly into the liver.

The thoracoabdominal incision utilized in this study in order to perform a high line of anastomosis undoubtedly is more traumatic to the animal than the subcostal approach employed by Child. This might also contribute to the increased mortality of the procedure in animals already in a border line state of adrenal insufficiency.

Table 1 Changes in Blood Ammonia in 5-6 Hour Period

GROUP	NO OF DOGS USED	FEEDINGS	NO OF OBSERVATIONS	MEAN CHANGE IN BLOOD AMMONIA	STANDARD ERROR OF DIFFERENCE OF MEANS
1	4 Normal	1000 cc Blood	4	-4%	±30 S D
2	3 Eck Fistula Milk and Mash		12	+48%	±12 S D
3	3 Eck Fistula 200 cc Blood		12	+16%	±376 S D Group 1 114
4	3 Eck Fistula 400 cc. Blood		7	+193%	±147 S D Group 2-4 662
5	2 Eck Fistula 700 cc Blood		14	+314%	±296 S D Group 2 5 792 Group 1 5 797

ml of blood following the afternoon blood ammonia determination. A period of 18 hr was then allowed to elapse before the next determination. Thus each dog received a total of 1050 ml of blood over a 24 hr period. A series of 10 observations was recorded in this phase of the experiment.

RESULTS

Table 1 records the results of the blood feeding experiment. It indicates that in normal dogs the feeding of 1000 ml of blood produces no real change in the blood ammonia level at the end of a 5 to 6 hr period. The change of minus 4% found in this group represents only an arithmetical mean change and is so slight as to be impossible to measure accurately with present techniques. When Eck fistula dogs were fed milk and mash a mean rise of +48% resulted at the end of a 5 to 6 hr period. Thus we have interpreted as representing the mean change occurring in Eck fistula dogs 5 to 6 hr after they have consumed a standard diet. When the Eck fistula dogs were fed 200 ml of blood a mean rise of 165% resulted. However this rise was not significant when compared with the mean change found after feeding milk and mash using twice the standard error of the difference of the two means as a measure of significance. When 400 ml of blood were fed to Eck fistula dogs a rise of 193% resulted. This rise was significant when compared with the mean rise seen following the milk and mash diet. When 700 ml of blood were administered to Eck fistula dogs the mean rise of 314% compared with the rise of 48% seen following the milk and mash feedings demonstrated most clearly that a significant rise in the blood ammonia could be produced by the digestion of blood in the gastrointestinal tract.

Table 2 represents the results of feeding blood to Eck fistula dogs with resultant blood ammonia changes in relation to time. When the dogs were fed 700 ml of blood a mean rise of 314% was found at the end of a 5 to 6 hr period. If these same dogs were given an additional 350 ml of blood at the end of the 5 to 6 hr period and a period of 18 hr was allowed to elapse before the next blood ammonia determination it was found that a fall occurred during the 18 hr period almost equalling the rise that occurred in the 5 to 6 hr period earlier in the day. Thus the mean change in the blood ammonia level over a 24 hr period during which time a total of 1050 ml had been given was only 31.9%. Many of our Eck fistula animals exhibited this characteristic of being able to effect a reduction in the blood ammonia level to baseline or prefeeding level if allowed sufficient time between feedings. However there were 2 exceptions to this observation.

DIGESTION OF BLOOD IN THE GENESIS OF HEPATIC COMA*

HAROLD F. WITCH, JOSEPH C. PINDER AND JOHN E. KIRBY

The observation that hepatic coma in patients with cirrhosis is so often associated with the onset of esophageal bleeding has suggested to us that the bleeding itself must be directly responsible for the initiation of the coma. Previous investigators have shown that a diet rich in protein will, in some cirrhotic patients, evoke the signs and symptoms of hepatic coma.¹ It also has long been known that one can produce meat intoxication in Eck fistula dogs by giving them in excess of animal protein. Thus it is not unreasonable to assume that blood which contains 175 gm of protein/l may when introduced into the bowel in a hemorrhage furnish sufficient nitrogen to cause hepatic coma in patients with severe liver disease. In order to test this hypothesis in dogs with deranged liver function we prepared a series of Eck fistula dogs and then fed them varying quantities of blood and observed their responses to these feedings and the changes produced in their blood ammonia levels.

METHOD

Using an end to side portocaval shunt we produced an Eck fistula in 7 dogs and then fed these dogs 200 to 700 ml of banked outdated human blood through a gastric tube. Blood ammonia determinations were carried out twice daily using the method of Conway: the first in the midmorning the second in the mid-afternoon approximately 5 to 6 hr after the morning value. The dogs were fed shortly after the determination of the morning blood ammonia level. If the feedings were less than 300 ml the blood was given in 1 feeding; if the amount was greater than 300 ml, the total amount was given in 2 feedings an hour apart. This was done to reduce the incidence of vomiting which was common when larger amounts were given.

A total of 21 observations was recorded. Each represented a feeding with the resultant change in the blood ammonia level. There were 12 observations regarding the changes following the feeding of 200 ml of blood, 7 following the feeding of 100 ml of blood and 11 following the feeding of 700 ml of blood. As controls 4 normal dogs were given 1000 ml of blood in two 500 ml feedings with the first feeding given shortly after the morning ammonia determination. Thus, as in the Eck fistula dogs a period of 5 to 6 hr elapsed between the 2 determinations. A total of 14 observations were carried out on the control dogs.

Three Eck fistula dogs were fed a diet of milk and mush in order to determine the daily variations in blood ammonia levels occurring in animals on a standard diet. Blood ammonia levels were determined on the same schedule as for the preceding groups of animals. A series of 12 observations was recorded for this group.

In order to assess further the time factor in relation to the feedings and to the changes in the blood ammonia levels those 2 dogs to whom 700 ml of blood had been administered in the morning were given an additional 350

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Table 3 Clinical Cases

	SURVIVED	DIED	DIED WITH BOTH CONTINUING HEMORRHAGE AND COMA	DIED WITH CONTINUING HEMORRHAGE BUT WITHOUT COMA	DIED IN COMA AFTER HEMORRHAGE STOPPED	DIED FROM CAUSES OTHER THAN CONTINUING HEMORRHAGE OR COMA
19 Cases Without Elevated Blood Ammonia	9	10	1	7	0	2
22 Cases With Elevated Blood Ammonia	3	19	12	0	0	7

As a clinical corollary to these experimental observations the progress of 35 consecutive patients with liver disease and alimentary tract hemorrhage was followed in the hospital. These cases are analyzed in Table 3.

There were 19 patients without elevated blood ammonia levels. Two of the 10 deaths in this group were attributed to causes other than coma or hemorrhage while the remaining 8 patients died of exsanguination. Only 1 of these patients was comatose at the time of death. Of the 22 patients with elevated blood ammonia levels only 3 survived. Seven of the 19 deaths in this group were from causes other than hemorrhage or coma. Several deaths were related to operative intervention for the purpose of hemostasis. All 12 of the remaining patients died with continuing hemorrhage and coma. It is especially noteworthy that in this series continued hemorrhage was the commonest cause of death and no patient died in coma once the bleeding was controlled.

CONCLUSIONS

These observations on animals and patients support the hypothesis that the coma often observed following gastrointestinal hemorrhage in severe liver disease is frequently caused by ammonia intoxication. This ammonia intoxication results from inability of the diseased liver to metabolize in a normal manner ammonia derived from the protein of the blood lost into the gastrointestinal tract. Stopping the hemorrhage is thus an important factor in the prevention of this type of hepatic coma.

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Table 2 Blood Ammonia Changes in Relation to Time

AMOUNT OF BLOOD FED	NO OF OBSERVATIONS	TIME ELAPSED AFTER FEEDING	MEAN CHANGE IN BLOOD NH ₃ N LEVELS
700 ml	14	5-6 Hr A M — I M	+314%
900 ml	10	18-19 Hr I M — A M	-108%
1000 ml	10	24 Hr A M — A M	+317%
AVERAGE A M BLOOD NH ₃ N BEFORE FEEDING 278% (4 Obs)			
AVERAGE A M BLOOD NH ₃ N AFTER FEEDING 1000 ml/day 288% (10 Obs)			

The first exception was those Eck fistula dogs who maintained continuously high blood ammonia levels on a low protein diet. These animals appeared chronically ill and if fed protein even in minute amounts would exhibit elevations out of proportion to the response expected in Eck fistula animals. More significant however was the observation that in these animals the fall in the blood ammonia level did not equal the rise caused by the feeding but that each feeding usually would cause a progressive rise in the baseline blood ammonia level. These Eck fistula dogs rapidly developed signs and symptoms of meat intoxication while those animals in whom the fall in the blood ammonia equalled or nearly equalled the rise leaving the baseline virtually unchanged at the end of a 24 hr period usually did not develop signs and symptoms of meat intoxication even though the blood ammonia level often rose to 4 or 5 times the prefeeding baseline level at some time during the 24 hr period.

The second exception apparent from earlier meat feeding experiences was that by spacing the feedings over short periods of time we were able to overwhelm the animals with excessive amounts of protein. In this manner it was possible to sustain a fairly high blood ammonia level in many of the Eck fistula dogs.

Two Eck fistula dogs died as the result of digestion of blood in the bowel. Both animals had markedly elevated blood ammonia levels and exhibited the characteristic signs and symptoms of meat intoxication. The first animal which had a chronically elevated blood ammonia was fed 1200 ml of blood in 6 feedings over a 36 hr period with the deliberate intention of producing coma. The dog died in coma 14 hr after the first feeding with a blood ammonia level of 840%. The second animal was an Eck fistula dog who had not been fed blood. Previously in good health this dog approximately 34 hr before its death developed progressive signs and symptoms of meat intoxication and gradually lapsed into coma and died in coma with a blood ammonia level of 780%. The dog received transfusions and fluid therapy for 12 hr preceding its death and did not appear to be at any time in a state of shock. Autopsy revealed a patent portacaval shunt with the stomach and small bowel filled with blood. The source of the hemorrhage was numerous small ulcerations of the gastric mucosa. No other factors contributing to the animal's death could be found.

as described by Harper *et al*.⁷ Samples of blood were drawn immediately before the infusion of glycine and at 30 min intervals thereafter, for a 2 hr period. Heparin was used as the anticoagulant. The samples of blood were analyzed for ammonia nitrogen by the microdiffusion method of Conway⁸ and for urea nitrogen by the method of Van Slyke and Kugel.⁹

The effect on blood ammonia of arginine or of monosodium glutamate was evaluated by two types of experimental procedures.

1. Infusions of monosodium glutamate or of arginine were administered at a rate of 1.75 mg amino N/kg/min concurrently with glycine at a rate of 3.5 mg amino N/kg/min and the levels of ammonia in the blood were compared with those obtained during the infusions of glycine alone.

2. Concentrations of ammonia sufficient to produce toxicity were allowed to accumulate in the blood as a result of continuous infusions of glycine at 3.5 mg amino N/kg/min for 1 hr at which time the glycine infusion was continued at the same rate but 25 gm of arginine or 50 gm of monosodium glutamate were added and the mixture was administered for an additional hour.

RESULTS

The infusion of glycine alone produced a marked rise in blood ammonia but when arginine was administered together with glycine this rise was prevented. In fact at the termination of the injection the reduction of blood ammonia over that of the control was tenfold. Under the same experimental conditions monosodium glutamate was able to effect only a slight decrease in blood ammonia. A comparison of the concentrations of ammonia in the blood after infusion of glycine alone, glycine with monosodium glutamate and glycine with arginine are shown in Figure 1a.

The changes in the urea nitrogen of the blood that followed injection of arginine when compared to those after infusion of monosodium glutamate suggested that the superior effect of arginine in preventing a rise in ammonia was due to increased production of urea (Fig. 1b).

The levels of ammonia and urea in the blood that occurred after the delayed injection of arginine or of monosodium glutamate are shown in Figures 1c and 1d. In these experiments the ability of arginine to bring about a reduction in the blood ammonia was clearly shown. Under the same conditions however monosodium glutamate proved ineffective although given at twice the dose of arginine.

The ability of arginine to decrease significantly or to prevent a rise in blood ammonia levels under experimental conditions suggested a trial of this agent in clinical disease states associated with elevated blood ammonia levels. The effect of intravenous administration of arginine was therefore studied in 20 patients with elevated blood ammonia levels and varying degrees of encephalopathy. In every instance a reduction in the levels of ammonia in the blood occurred following arginine therapy. The most satisfactory results were obtained in cases with acute ammonia intoxication secondary either to massive hemorrhage or to ammonium chloride ingestion. In those patients with chronically elevated blood ammonia the response was somewhat more delayed. However in each instance improvement in the mental status of the patient accompanied the reduction of blood ammonia levels. In over half the cases the patients were initially in deep coma, how-

A COMPARISON OF ARGININE AND L-GLUTAMIC ACID IN THE TREATMENT OF AMMONIA TOXICITY*

JOHN S. NAJARIAN AND HAROLD A. HARPER

The toxic effects that follow the experimental elevation of ammonia in the blood in dogs have been described by Koprowski and Uninski.¹ Disturbances of the central nervous system similar to those which occurred in the experimental animal after the administration of ammonium salts have also been reported in patients with elevated levels of ammonia in the blood.² These findings have stimulated increased interest in the possible role of ammonia in clinical disease.³

The problem is important to the surgeon because two of the most common clinical syndromes associated with elevated levels of ammonia in the blood may occur either in patients who have had vascular shunting procedures performed to ameliorate portal hypertension or in those in whom chronic liver disease is associated with gastrointestinal hemorrhage.

The treatment of patients with elevated blood ammonia levels requires the use of measures to control the amount of ammonia derived from the gastrointestinal tract as well as efforts to reduce its concentration in the blood. The production of ammonia in the gastrointestinal tract can be controlled by (1) limitation of the intake of protein, (2) control of gastrointestinal bleeding and prompt removal by catharsis or lavage of blood which may have accumulated from hemorrhage and (3) oral administration of antibiotics in an effort to diminish the activity of the intestinal bacteria which produce ammonia from nitrogenous substrates. The administration of ammonium salts or of resins containing ammonia must also be avoided in these patients.

Reduction of the concentration of ammonia in the blood may be accomplished by enhancement of the reactions by which it is normally metabolized and removed from the body. These reactions include its excretion as ammonium salts or as urea or its detoxification by transamination. The rationale for the glutamic acid therapy of ammonia intoxication proposed by Walshe⁴ and McDermott⁵ is based on removal of ammonia by amidation of the glutamic acid to form glutamine. The formation of urea, however, is quantitatively the most important method by which ammonia is removed from the body. Experiments were therefore undertaken to lower blood ammonia levels by attempting to increase urea production.

Arginine is the amino acid which is the immediate precursor of urea in the Krebs-Henseleit cycle. The effect of arginine as well as that of glutamic acid on the blood ammonia was therefore studied under the same experimental conditions in order to evaluate the relative efficiency of urea production as compared to transamination for detoxification of ammonia.

METHOD

Ammonia intoxication was induced in adult mongrel dogs by rapid intravenous administration of glycine at a rate of 3.5 mg. of amino N/kg./min.

*From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco. Supported by the Christine Breon Fund for Medical Research.

ever with 1 exception all became alert and had a clear sensorium within 24 to 48 hr following arginine therapy

SUMMARY

1 The effects on the blood ammonia of simultaneous or delayed administration of either L arginine hydrochloride or L monosodium glutamate were studied in dogs

2 Arginine was much more effective than monosodium glutamate either in preventing a rise or in reducing the levels of ammonia in the blood

3 When arginine was given the rise in blood urea that accompanied the marked fall in blood ammonia indicated that this amino acid exerted its effect on the blood ammonia by its influence on the production of urea

4 Intravenously administered arginine was also found to be effective in lowering elevated levels of ammonia in the blood of patients with clinical manifestations of encephalopathy of varying degrees

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MORTALITY FACTORS IN EXPERIMENTAL HEMORRHAGIC PANCREATITIS*

IRVIN H SOKOLIC AND ALEX W ULIN

The factors responsible for the high mortality in acute hemorrhagic pancreatitis in man have been variously ascribed to 1) infection 2) a specific circulating toxic material of pancreatic enzyme origin 3) release of non specific toxic materials due to marked tissue necrosis 4) disturbed fluid and electrolyte balance and 5) reduced effective circulating blood and plasma

*From Dept of Surgery Hahnemann Medical School and Hospital and Albert Einstein Medical Center Southern Division Philadelphia Pa With the technical assistance of Dr John Kachmar Mr William Chambres and Miss Marcelle Rosenberg

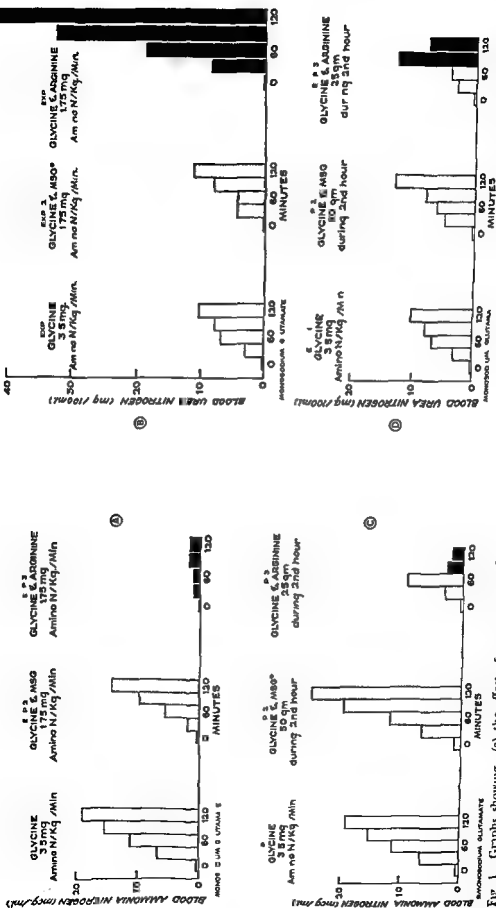


Fig 1 Graphs showing (a) the effect of intravenous infusion of various amino acid mixtures on the levels of ammonia in the blood (b) the effect of intravenous infusion of various amino acid mixtures on the levels of urea nitrogen in the blood (c) the effect of monosodium glutamate and arginine on elevated levels of ammonia in the blood (d) the effect of monosodium glutamate and arginine on elevated levels of urea nitrogen in the blood. Glycine was given for 2 hr in Experiment 1. In Experiment 2 glycine was given for the first hour and a mixture of glycine and monosodium glutamate was given during the second hour. In Experiment 3 arginine was added to the glycine during the second hour. (d) Blood urea nitrogen after administration of monosodium glutamate and arginine in the first hour of the experiment. The experiment was carried out in human beings.

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SUMMARY

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The factors responsible for the high mortality in acute hemorrhagic pancreatitis in man have been variously ascribed to 1) infection 2) a specific circulating toxic material of pancreatic enzyme origin 3) release of non-specific toxic materials due to marked tissue necrosis 4) disturbed fluid and electrolyte balance and 5) reduced effective circulating blood and plasma

*From Dept of Surgery, Hahnemann Medical School and Hospital and Albert Einstein Medical Center Southern Division Philadelphia Pa. With the technical assistance of Dr John Kachmar, Mr William Chambers and Miss Marcelle Rosenberg

volume. Each of these factors was investigated in turn by the techniques to be described below.

As a control for evaluation of further studies, acute hemorrhagic pancreatitis with necrosis was produced in 6 dogs by the method of Archibald,¹ namely by injecting 10 cc of gallbladder bile into the main duct of the pancreas using an injection forcible enough to produce duct rupturing. Rupture of the ducts was attested to by the fact that the bile tended to distribute itself throughout the pancreatic parenchyma and in the interlobular spaces. This was further confirmed by microscopic study. Intravenous nembutal and pentothal anesthesia was employed and postoperative care was limited to the supportive use of parenteral fluids when indicated. This routine was also followed in all cases to be described below. Serum amylase and lipase studies were also carried out. As has been demonstrated in the past, amylase values normally approximately 2,000 units in the dog rose to values between 12,000 and 32,000 Somogyi units. The typical gross lesions of necrotizing pancreatitis were seen on autopsy examination of the animals at the time of death. Bacteriologic study showed the Welch bacillus to be the most common pathogen. None of the 6 animals survived more than 72 hours.

The role of infection was then investigated by repeating and verifying the work of Fine^{2,3} and his group. Pancreatitis was produced in 3 animals by the methods described above. Two dogs were treated with intramuscular penicillin and streptomycin and 1 with intramuscular oxytetracycline. Antibiotics were given over a 4 day period. In each instance amylase values over 8,000 units were obtained but the animals survived. Autopsy examination after sacrifice 7 to 10 days after the induction of pancreatitis showed an edematous, firm pancreas. Hemorrhage into the gland and into the peritoneal cavity was not marked as in the previous preparations. These results verified the reports of previous investigators and were pursued no further. Thus it appeared that the prevention of infection was of paramount importance in reducing the mortality of bile injection pancreatitis.

The escape of specific circulating toxic enzymes with their alleged devastating effects on the body economy has long been thought to be a major factor in the poor prognosis of pancreatic necrosis. Investigation of this problem was approached in the following manner. It has been shown by others^{4,5} that the administration of dl ethionine causes marked histologic alterations of the pancreas. These are manifested during the first 5 days by loss of basophilia, vacuolization of the cytoplasm and apparent decrease in the amount of zymogen granules of the cells of the acini. Also during this time there is a steady fall in the level of peripheral serum enzymes and some evidence to indicate a fall in enzyme content of the pancreatic secretions. This has been interpreted to indicate that enzyme production of the pancreas is markedly altered and inhibited by the administration of ethionine. If these conclusions are correct, then there is afforded us a powerful tool for the study of bile injection pancreatitis, namely a pancreas partially devoid of enzyme content. Accordingly, 27 dogs were given ethionine in doses varying between 25 and 150 mg/kg for a period of 1 to 5 days. A typical protocol is reproduced below. (See Fig. 1.)

In only 2 instances did the animal pretreated with dl ethionine survive the pancreatic insult. Thus the mortality of hemorrhagic pancreatitis is not significantly altered by modifying the enzyme content of the pancreas. This

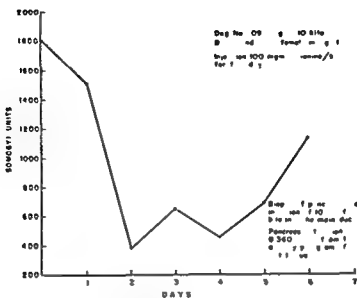


Figure 1

would suggest that the release of pancreatic enzymes is not an important factor in contributing to the high mortality of pancreatitis.

The evaluation of the release of non specific toxins from the necrotic tissues was next undertaken. With a dog under nembutal anesthesia 10 cc of bile was injected into the main pancreatic duct producing the usual marked edema of the pancreas. The entire pancreas was then excised, placed in a Waring blender and homogenized. To the homogenate was added 100,000 units of penicillin and 0.5 gm of streptomycin. The entire tissue mash was then injected into the peritoneal cavity of a second animal through a trocar incision. All 3 animals which were injected did well clinically in the immediate postoperative period. Amylase values of 5,000 to 9,000 units were recorded yet the animals were alert, ate and walked about with none of the stigmata of abdominal catastrophe. This indicated to us that the release of nonspecific breakdown products was probably not of importance and this approach was abandoned.

The evidence gathered to this point then seemed to indicate that there was nothing inherent within the acutely inflamed pancreas which was in itself lethal. The problem of hemorrhagic pancreatitis appears to have evolved itself into an appraisal of the general systemic effects produced by the localized pancreatic disease. To test the effect of localized pancreatic disease in parts of the body other than the peritoneal cavity the following technique was devised. A series of dogs was submitted to a 2 stage procedure wherein during the first stage the pancreas was split into 2 parts in the manner shown in the following diagram (See Fig 2).

In control animals the distal pancreas was left within the peritoneal cavity and in test animals it was planted into a subcutaneous pouch. Seventeen animals survived the procedure. In 6 control animals hemorrhagic pancreatitis was then produced by bile injection at a second operation 2 to 3 wks after the original procedure. Five of the animals died with postmortem evidence of hemorrhagic pancreatitis and peritonitis.

In the 11 test animals the proximal half of the pancreas was excised during the first stage in 8 dogs and allowed to remain in place in 3 dogs. Hemorrhagic pancreatitis was then produced in the extraperitoneal pancreas by the

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THE ROLE OF PROPYLTHIOURACIL IN PANCREATITIS*

L. CORSAN REID THOMAS W. CHALIS ROBERT L. PAULFETTE
 AND J. WILLIAM HINTON

Recently it has been shown^{1, 2} that thiourea and its analogues owe their anti-thyroid action in part at least to their antioxidant property in all organs and tissues. In other words they interfere with the utilization of oxygen in all organs and tissues. As a consequence there is a decrease in the amount of energy released, less product is formed and in the case of the thyroid less thyroxine is produced. In addition the restriction in the amount of oxygen utilized by all other organs and tissues results in a marked lowering of the basal metabolic rate. Propylthiouracil has been a particularly active compound in bringing about these changes.

Starr stated at a meeting of the Pan Pacific Surgical Association in Honolulu in October 1951 that propylthiouracil was a valuable therapeutic agent in acute pancreatitis. No comments were made about its mechanism of action. As a result of this statement and our experience with propylthiouracil the following working hypothesis was evolved which was susceptible of being tested experimentally. The results of this investigation constitute the basis of this report.

Propylthiouracil inhibits oxygen utilization in the pancreas; less energy is released by oxidative cell energy mechanisms; less work is done; less products are produced and the organ is placed at rest when under severe stress. This decreased synthetic activity should permit a larger proportion of the energy released in the organ to be available for the maintenance of the morphological integrity of the cells of the pancreas. This is of the utmost importance in preventing the escape of a flood of powerful proteolytic enzymes which bring about almost instantly destructive and irreversible alterations in cell organization and organ structure. These particular intracellular changes occur only in the pancreas. This makes the preservation of the cell structure of the pancreas vastly more important than that of any other organ.

In addition the effect of propylthiouracil in lowering the metabolic tempo of all the other organs should serve to decrease further the demands on the pancreas.

METHOD

1. The hypothesis was tested by measuring the effects of propylthiouracil upon the oxygen uptake of slices, minced homogenates and mitochondria.

*From the Department of Surgery, New York University Post Graduate Medical School, New York. Supported by the Ruth H. Kres. Grant to the John F. Edlmann Surgical Research Laboratory.

from the pancreas and comparing these findings with similar preparations under the same conditions with other organs such as liver and kidney by the usual manometric techniques

2 The average serum amylase levels of normal dogs were established in a large number of animals and compared to animals receiving propylthiouracil

3 Dogs receiving ACTH were treated with propylthiouracil

RESULTS

Tissue slices and tissue mince from the pancreas showed moderate oxygen uptake. Homogenates and mitochondria on the other hand showed practically no oxygen consumed under standard conditions. On addition of methylene blue acceptable readings were obtained but these were significantly lower than those found in liver or kidney. On addition of propylthiouracil in increasing quantities there was a progressive linear decrease in oxygen uptake. This was true of all homogenates but was less obvious in the case of the pancreas than other organs.

Table 1

ORGAN	CONTROL	MICROMOLES PER ML. OF HOMOGENATE						
		2.4	4.8	7.2	9.6	12.0	14.4	16.8
KIDNEY	1236	1072	936	714	571	457	350	292
	1132	1065	892	672	564	432	347	273
LIVER	975	917	884	801	729	602	503	426
	974		865	793	724	586	476	380
PANCREAS	375	366	370	311	—	280	227	177
	373	352	332	267	—	216	226	161

In the case of the mitochondrial preparations all were exquisitely sensitive to propylthiouracil and in the case of the pancreas this was particularly so (Fig. 1).

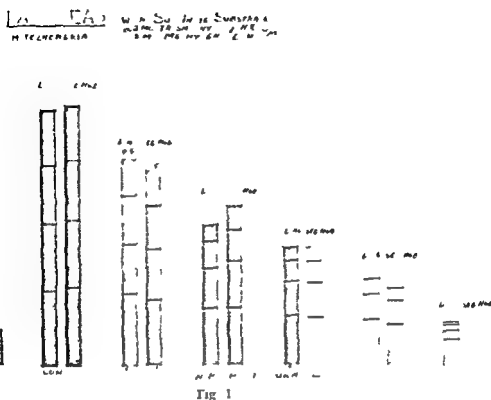
Eighteen dogs received 300 mg propylthiouracil/day for an average of 2 wks. All but 3 showed a marked decrease in the serum amylase level and in 2 of the 3 failures a significant decrease occurred on increasing the dose of propylthiouracil.

Propylthiouracil 75 mg/day was given to 5 dogs on ACTH. The results were equivocal.

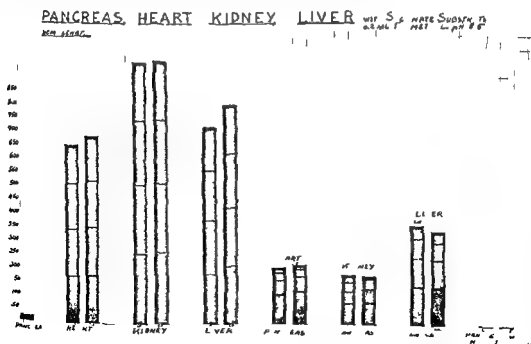
DISCUSSION

The oxygen uptake by slices and mince can be satisfactorily explained on the basis that cell organization is relatively intact, energy release mechanisms are operating and oxygen is consumed.

The failure of homogenates and mitochondria to utilize oxygen appears to depend upon the almost instantaneous disorganization of the cell machinery as a result of the rupture of the cell membrane. The addition of methylene blue to the Warburg vessels greatly improved the oxygen utilization in homogenates and mitochondria which was not true under similar conditions in either liver or kidney.



The inhibition by propylthiouracil of oxygen uptake in pancreatic homogenates was not as marked as in other organs and this may well be due to the results of the action of degradative enzymes. In the case of the mitochondria less structural disorganization has occurred and they are as sensitive as those from other organs to smaller dosages of the drug.



When one recalls that 85 per cent of the energy released by the cell is an oxidative process which is exclusively localized in the mitochondria these effects of propylthiouracil upon pancreatic mitochondria take on considerable significance and would appear to have therapeutic possibilities.

The rapid and powerful proteolytic activity of the intracellular pancreatic enzymes is clearly shown by the almost complete inhibition of oxygen uptake when one adds pancreas homogenates to liver or kidney homogenates. These proteolytic pancreatic enzymes are equally destructive to liver and kidney as well as pancreas (Fig 2). In mixing together any other 2 organ homogenates no such changes are seen.

In the 18 dogs receiving 300 mg propylthiouracil/day the lowering of the serum amylase level indicates an inhibition of protein synthesis regardless of the source of serum amylase.

Previous work from this laboratory brings rather convincing evidence that the release of ACTH plays a dominant role in the elevation of the serum amylase and the serum amylase level is appreciable even in pancreatectomized dogs with total removal of the salivary glands.

On giving 75 mg propylthiouracil/day to 5 dogs on ACTH injections the lack of any clear cut findings may be due to the low dosage employed.

SUMMARY

1 Propylthiouracil inhibits oxygen uptake with the result that there is a reduction in the energy supply available to the organ as shown in its action on homogenates and mitochondria from the pancreas.

2 Propylthiouracil lowers the serum amylase levels in normal dogs.

3 Present experience indicates that propylthiouracil will modify the elevation of serum amylase that results from the release of ACTH under stress.

4 These experimental findings suggest that propylthiouracil reduces the tempo of pancreatic metabolism by its direct anti-oxidant action on the pancreas itself and indirectly by a similar action on all other body organs and tissues. This serves to place the organ at rest when under stress. This should be a therapeutically useful procedure in pancreatitis by making more energy available for maintaining the anatomical and cellular integrity of the organ at the price of lowered functional activity.

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DIAGNOSTIC VALUE OF URINARY LIPASE*

ALAN D. CALLOW, MARTIN M. NOTHMAN AND JOSEPH H. PRATT†

Because studies on fat splitting urinary enzymes are rare and results are contradictory, this investigation has attempted to confirm the existence of such an enzyme in urine to demonstrate that its chief source is the pancreas and to consider its usefulness as a diagnostic aid.

METHOD

Utilizing a method previously described¹ a fat splitting enzyme has been regularly found in the urine of dogs.² A urine-olive oil emulsion mixture was incubated for 24 hr. for the titration of liberated fatty acids to a pink end point with phenolphthalein and N/20 sodium hydroxide. The amount of sodium hydroxide required for such titration usually 0.1 cc. to 0.75 cc. has been expressed as units of the enzyme. One hundred and fifty samples of urine from normal dogs were tested and values were found to range from 0.15 to 1.0 units. Injection of secretin or methacholine (Mecholyl) increased the amount of enzyme in the urine. Removal of the pancreas resulted in immediate disappearance of the enzyme in the urine. Ligation of the pancreatic ducts produced a marked increase in the concentration of the urine whereas ligation of the pancreatic ducts followed by pancreatectomy produced a rapid disappearance in the previously high urinary enzyme values. It has been concluded that the fat splitting enzyme is of pancreatic origin.

A fat-splitting enzyme has also been detected regularly in the urine of man.³ Utilizing the same method of titration of liberated fatty acids 500 samples of urine from healthy individuals yielded values which ranged from 0.1 to 0.75 units. Values of 1.0 units or more were considered significantly elevated. Urine samples collected at 2 hr. intervals for 24 and 48 hr. from male patients free of gastrointestinal signs and symptoms and who had received secretin and mecholyl by injection yielded characteristic values for the amounts of fat-splitting enzyme. Values regularly rose 2 hr. after secretin administration, reached a maximum in 6 to 8 hr. and returned to normal in 24 hr. The normal peak response was 1.2 units. No significant difference occurred in the values of urinary lipase in the same individual during the same day or on consecutive days. Values ranged between 0.2 and 0.45 units in 1 individual whose urine was tested 3 times daily for 7 days and in another individual varied from 0.35 to 0.55 units. There is apparently no change in the concentration of the urinary lipase as a result of fasting or ingestion of food. In 25 cases the total 24 hr. volume of urine was tested and revealed no significant variation on a day to day basis of urinary lipase. In 12 cases of pregnancy the volume of urinary lipase ranged again from 0.1 to 0.75 units. The patients tested were in their 3rd to 7th months of gestation.

Thirteen cases of carcinoma of the pancreas established by subsequent surgical exploration were found to yield normal values for urinary lipase when such patients did not have secretin or mecholyl stimulation. Injection

From the Departments of Medicine and Surgery, Tufts University School of Medicine and New England Center Hospital, Boston, Mass. Supported by the Damon Runyon Memorial Fund for Cancer Research.

†deceased.

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Tumor Problems and Transplantation

LOCALIZATION OF P^{32} AS A DIAGNOSTIC AID IN GASTROINTESTINAL NEOPLASMS*

DONALD B. SHAWON, J. BRADLEY AUST AND HARLAN D. ROOT

Neoplastic tissues because of their more rapid growth rate have been found to take up a measurably higher percentage of radioactive phosphorus than normal tissues.¹ Incorporation of phosphorus in the nucleoproteins of neoplastic tissue is several times greater than in normal tissues which have a slower rate of mitoses.^{2,3} This property of radioactive phosphorus (P^{32}) has had clinical application in the diagnosis of neoplasms of the breast,⁴ brain,⁵ and lung.⁶ We have applied this technique to the study of patients clinically suspected of gastrointestinal cancer who had undergone surgical resections of their involved organs.

METHOD

Radioactive phosphorus (P^{32}) has a half life of 14.3 days. Its maximum energy of beta radiation is 1.71 million electron volts (MeV), an average of 0.695 MeV, with a maximum tissue penetration of 0.8 cm. This is entirely beta radiation since there is no gamma radiation from the isotope.

The radioactive phosphorus (P^{32}) was obtained from the Oak Ridge National Laboratories. Approximately 0.5 mc. of P^{32} was injected intravenously 4 to 48 hr. prior to surgery in each of the 78 patients with suspected malignancies of the esophagus, stomach, colon, or rectum. Following removal of the diseased organ, each specimen was surveyed employing a mica window Geiger Muller tube 2 gm./cm.² in thickness obtained from the Anton Electronic Laboratories, New York City. A lead shield was placed around the window with an aperture of 0.8 cm. in diameter projecting 0.1 cm. beyond the window. The Geiger Muller tube was attached to a Berkeley Decimal Geiger Muller Scaler model 1000 II. Multiple 1 min. counts of the cancerous area and the adjacent normal mucosa were obtained for differential study. Background counts were obtained in each instance. The cancer:normal ratio of 1:3.1 or greater radioactivity as described by Nakayama⁸ was arbitrarily considered as being diagnostic of malignancy. Histologic verification of the diagnosis in the malignant and normal areas studied were obtained in all instances.

RESULTS

Seventy-eight patients were studied employing the method described above and the results are summarized in Table I. The differential counts of

*From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minn. Supported by Malignant Disease Research Fund, American Cancer Society Institutional Research Grant #19F, Jay and Rose Phillips Fund, Arthur and Stella Sanford Fund.

of secretin in these patients however resulted in a decrease in the concentration and in some cases the complete disappearance of the enzyme. There seemed to be no difference in response to secretin whether the tumor was located in the head of the pancreas (6 cases) or in the body or tail of the pancreas (7 cases). An explanation for this possible exhaustion phenomenon is not available and further studies are in progress.

A fat splitting enzyme apparently identical with pancreatic lipase of the dog has been regularly detected in the urine of man. Variations in the amount of urinary fat splitting enzymes by the same individual are very slight although variations in amount between different individuals do occur within limitations. Changes in the rate of urinary excretion of a fat splitting enzyme apparently occur in the diseased pancreas. Further studies are required for the interpretation of these changes.

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Neoplastic tissues because of their more rapid growth rate have been found to take up a measurably higher percentage of radioactive phosphorus than normal tissues.¹ Incorporation of phosphorus in the nucleoproteins of neoplastic tissue is several times greater than in normal tissues which have a slower rate of mitoses.^{2,3} This property of radioactive phosphorus (P^{32}) has had clinical application in the diagnosis of neoplasms of the breast,⁴ brain⁵ and lung. We have applied this technique to the study of patients clinically suspected of gastrointestinal cancer who had undergone surgical resections of their involved organs.

METHOD

Radioactive phosphorus (P^{32}) has a half life of 14.3 days. Its maximum energy of beta radiation is 1.71 million electron volts (MeV) an average of 0.695 MeV with a maximum tissue penetration of 0.8 cm. This is entirely beta radiation since there is no gamma radiation from the isotope.

The radioactive phosphorus (P^{32}) was obtained from the Oak Ridge National Laboratories. Approximately 0.5 mc of P^{32} was injected intravenously 4 to 48 hr prior to surgery in each of the 78 patients with suspected malignancies of the esophagus, stomach, colon or rectum. Following removal of the diseased organ each specimen was surveyed employing a mica window Geiger Muller tube 2 gm/cm² in thickness obtained from the Anton Electronic Laboratories, New York City. A lead shield was placed around the window with an aperture of 0.8 cm in diameter projecting 0.1 cm beyond the window. The Geiger Muller tube was attached to a Berkeley Decimal Geiger Muller Scaler model 1000 B. Multiple 1 min. counts of the cancerous area and the adjacent normal mucosa were obtained for differential study. Background counts were obtained in each instance. The cancer:normal ratio of 1:3.1 or greater radioactivity as described by Nakagawa⁶ was arbitrarily considered as being diagnostic of malignancy. Histologic verification of the diagnosis in the malignant and normal areas studied were obtained in all instances.

RESULTS

Seventy eight patients were studied employing the method described above and the results are summarized in Table I. The differential counts of

*From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minn. Supported by Malignant Disease Research Fund, American Cancer Society Institutional Research Grant #195, Jay and Rose Phillips Fund, Arthur and Stella Sanford Fund.

Table 1 Results of P³² Uptake in Resected Gastrointestinal Lesions

ORGAN	DIAGNOSIS	NO OF PATIENTS	POSITIVE DIAGNOSIS*	PER CENT CORRECT
Esophagus	Epidermoid carcinoma	6	6	100.0
Stomach	Adenocarcinoma (polypoid ulcerative and superficial spreading)	32	32	100.0
	Linitis plastica	5	0	0.0
	Benign gastric ulcers	7	3	42.9
Colon	Adenocarcinoma	15	15	100.0
	Benign polyps	1	1	100.0
	Benign ulcers	1	1	100.0
Rectum	Adenocarcinoma	11	10	90.9

*Counts higher than 13.1 equalled positive diagnosis of cancer

cancer normal in all cases with histologically proven malignancy ranged from 12.1 to 13.5. The counts in all patients with cancer of the esophagus ranged between 16.1 and 45.1.

Cancer normal counts in 37 patients with cancer of the stomach ranged from 13.1 to 13.5. In 32 specimens all of which demonstrated grossly and microscopically, polypoid ulcerating or superficial spreading carcinomas. Approximately 60 per cent of this group revealed differentials of 20.1 or higher. One patient, a 65-year-old female, underwent exploratory laparotomy for symptoms of persistent epigastric distress and achlorhydria. Roentgenologic examination of the stomach had failed to reveal any lesion. At surgery a very slight thickening in the antrum was the only abnormality noted. A subtotal gastrectomy was performed. Gross examination of the resected stomach by the pathologist failed to reveal any lesion. The specimen was then surveyed by Geiger-Müller tube which resulted in localizing an area on the anterior wall of the antrum which counted 15 times the normal. Microscopic sections taken through this area demonstrated a minute and very early superficial spreading carcinoma of the stomach.

Multiple benign gastric polyps were noted in 1 patient with an associated carcinoma of the stomach. Differential counts of 13 to 14.1 were obtained in the nonmalignant polyps with no differentiation of the polyps from the carcinoma.

Diagnostic counts were unobtainable in 5 patients with linitis plastica. Diffuse submucosal involvement of the entire resected specimen without ulceration was noted microscopically in all 5 specimens. Due to the absence of any normal stomach for differentiation from the cancer, diagnostic counts were not obtainable.

Four of the 7 specimens with histologically proven benign gastric ulcers showed differential counts ranging from 14 to 21.1. All demonstrated acute inflammatory changes or fibroblastic activity in the involved area. Since phosphorus is taken up to a greater degree by rapidly growing tissues, the increased cellular activity noted in these lesions characterized by inflammatory and fibroblastic activity could account for the high uptake in these 4 instances.

Differential counts in 16 colon specimens with microscopic verification of carcinoma ranged from 1:3:1 in 1 patient to 9:2:1 in the remaining 15. Cancer normal ratios of 2:0:1 or higher were noted in over 60 per cent of this group. Two colon specimens with nonmalignant lesions (one—multiple benign polyps and the other—superficial nonmalignant ulcerations) had count ratios less than 1:3:1. In 1 instance of carcinoma of the descending colon an associated villous papilloma in the upper sigmoid was encountered. Differential counts of this lesion were similar to those in the cancer. Microscopic examination of the papilloma failed to reveal any malignant change.

An interesting differential count was obtained in 1 patient with multiple polyps of the sigmoid colon. One polyp had been previously biopsied and diagnosed microscopically as adenocarcinoma. Examination of the resected specimen revealed several polyps ranging from 0.3 cm. to 2.0 cm. in diameter. Differential counts of several of the polyps revealed 2 to record high ratios of 2:9:1 with the remaining polyps failing to demonstrate any differentiation. These 2 polyps both proved to be malignant while those with no differentiation failed to demonstrate microscopically any malignant changes.

Cancer normal ratios of the 11 patients with cancer of the rectum ranged from 1:2:1 in 1 patient to 3:3:1 in the remaining 10 patients. Differential counts of 2:0:1 or greater were observed in 5. Excluding those patients with linitis plastica the only specimen with proven malignancy that failed to demonstrate a cancer normal ratio of 1:3:1 or greater was encountered in this group. Gross observation of this specimen revealed an ulceration in the distal rectum approximately 5 cm. by 3 cm. in diameter. Microscopic examination revealed the tumor cells to be well differentiated with no marked increase in mitoses.

Radioautographs of the resected specimens were obtained. Figure 1 demonstrates a positive radioautograph diagnostic of a gastric cancer. The dark



Fig. 1. Radioautograph of carcinoma of stomach.

ened area shows the radioactive phosphorus uptake in the region of the carcinoma

The use of minute Geiger Muller tubes passed through the oral cavity into the esophagus and stomach for surveying these organs *in vivo*, in order to differentiate carcinoma from normal tissue has been initiated. This is a report in progress and at present there are insufficient studies to warrant any definite conclusions.

DISCUSSION

The results obtained from this study have confirmed the reports of other investigators: malignant neoplastic tissue incorporates phosphorus in greater quantity than normal tissue. This was noted in all cancerous specimens studied, excluding 1 specimen with a carcinoma of the rectum. The 5 cases of linitis plastica failed to demonstrate any normal tissue for differentiation from the cancer. False positive determinations were noted in 6 instances: 1 with benign gastric ulcers, 1 with villous papilloma of the rectum, and 1 with multiple gastric polyps. These findings can be ascribed to the increased cellular activity as compared to the normal tissues in these organs. The use of P^{32} as an additional means of localization and diagnosis in gastrointestinal neoplasms appears warranted. Surveying of the more accessible organs *in vivo* such as the esophagus, stomach, and rectum by means of minute Geiger Muller tubes passed into these viscera would be of immeasurable value for localization and diagnosis of neoplasms. Differential counting at surgical exploration could enhance the performance of a more curative operation by localization of lymph node spread.

SUMMARY

1. Seventy-eight surgically resected specimens of patients with suspected gastrointestinal malignancies were studied employing P^{32} administered intravenously. A cancer:normal ratio of 1:3:1 was the criteria accepted for diagnosis of malignancy.

2. The method of administration and counting are described.

3. Excluding 5 cases of linitis plastica in which differentiation between cancer and normal tissue was not possible due to the absence of adjacent normal stomach, adequate localization of radioactive phosphorus in the neoplasm failed to occur in only 1 instance. False positive counts suggesting a diagnosis of cancer were noted in 4 of 9 patients with benign gastrointestinal lesions.

4. The application of this technique as an aid in the diagnosis, localization, and surgical treatment of gastrointestinal neoplasms is discussed.

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FURTHER OBSERVATIONS ON THE LYMPHATIC PICK UP OF RADIOACTIVE SILVER COATED GOLD COLLOID ADMINISTERED INTRATHORACICALLY TO DOGS*

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AND G. R. MENFELS

In a previous publication¹ this laboratory reported that radioactive silver coated gold colloid injected into the empty hemithorax of pneumonectomized dogs caused the destruction of the superior mediastinal nodes in 90 per cent of those animals studied. No harmful effects from this treatment were noted. In this communication these results will be reviewed along with the results obtained by the mediastinal injection of the colloid at the time of pneumonectomy and following the intrapleural administration of the drug at the time of lobectomy.

METHOD

In these experiments mongrel dogs (weighing 10 to 19.5 kg) were used. They were operated upon under aseptic conditions using intravenous barbitalurate anesthesia. At operation or at various times during the postoperative period radioactive silver coated gold colloid was injected into the pleural space or mediastinum. The drug was obtained from the Brookhaven pile and processed after the method of Hahn¹ by the Abbott Laboratories of Chicago, Illinois. Previous studies² showed that it was necessary to use a dose of 40 mc or more in order to produce a consistent radiological effect on the mediastinal nodes. The interval between operation and injection of the isotope did not alter the results. The dogs were sacrificed at intervals ranging from 2 to 44 days after injection of the colloid material. At the time of sacrifice representative lymph nodes were dissected out, cleared of adventitial tissue, weighed and placed in standard formaldehyde tubes and the radioactivity determined by a Texas well counter. Histologic studies of the right bronchial, the carinal, the left bronchial, the anterior superior medi-

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ened area shows the radioactive phosphorus uptake in the region of the carcinoma

The use of minute Geiger Muller tubes passed through the oral cavity into the esophagus and stomach for surveying these organs *in vivo*, in order to differentiate carcinoma from normal tissue has been initiated. This is a report in progress and at present there are insufficient studies to warrant any definite conclusions.

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Table 2 Group 2 Dogs Receiving Mediastinal Infiltration of Gold Colloid

NUMBER	DOSE	β SUPERIOR ANTERIOR MEDIASTINAL	β SUPERIOR MEDIASTINAL LEFT	β SUPERIOR MEDIASTINAL RIGHT
1	50 mc	51 700	12 015	18 111
6	51 mc	51 100	16 700	16 9 800
7	51 mc	1,200	32 000	76,200
8	51 mc	69 000	10 500	2, 100
9	19 mc	15 700	2 000	12 900
10	49 mc	76 800	90 100	81 900
11	19 mc	58 000	116 900	76 100
13	50 mc	50 700	299,500	102 800
15	43 mc	109 700	115 800	21 300
18	50 mc	16,500	79 200	51 300
19	100 mc	53 800	8,200	204 500
22	50 mc	40 100	184 000	114 400

The residual lung was inflated and the chest was closed without drainage. These dogs were sacrificed from 7 to 10 days following the procedure. Nine of the 10 dogs (90 per cent) had significant irradiation of the superior mediastinal group of nodes as determined by the Texas well counter (Table 3). On histologic study, all the superior mediastinal nodes showed varying degrees of radiation effect. There was 1 death in this series. Dog No. 21 died on the fourteenth postoperative day following a left upper lobe lobectomy. At autopsy, pneumonia was present in the left lower lobe and there was atelectasis on the contralateral side. It was felt that this dog died as the result of the procedure but not as the result of the gold therapy. Except in the dog that died, the remaining lobe on the operated side was free of abnormalities at the time of autopsy.

Table 3 Group 3 Dogs Receiving Intrapleural Gold Colloid at Time of Lobectomy

NUMBER	DOSE	β SUPERIOR ANTERIOR MEDIASTINAL	β SUPERIOR MEDIASTINAL LEFT	β SUPERIOR MEDIASTINAL RIGHT
12	50 mc	55 400	25 500	19 900
14	50 mc	121,500	251 900	Count too near background count
16	43 mc	81 000	93 100	40 800
17	50 mc	Count too near background count	Count too near background count	6 100
20	50 mc	112 600	110 900	180 100
21	50 mc	191 400	11 300	36 300
23	50 mc	223 800	84 000	256 600
24	50 mc	91 100	26 500	30,200
25	35 mc	177 000	557 000	389 700
26	33 mc	290 000	219 000	381 300

astinal the right superior mediastinal and the left superior mediastinal lymph nodes were carried out. Previous studies² revealed that a significant quantity of the colloid material was present in the mediastinal nodes of dogs sacrificed on the second postinjection day; dogs sacrificed later did not have significantly more gold in the nodes studied. Therefore, in calculating the beta equivalent roentgens it was assumed that irradiation emitted after 48 hr. could be considered as the lymph node dose.

RESULTS

Of the 10 dogs in Group 1 that received more than 40 mc of the drug following pneumonectomy (Table 1) it is notable that the superior mediastinal group of nodes received adequate irradiation in 70 per cent of the cases. In 2 dogs the interval between injection and counting was so long that the counts were not reliable. Histologic study of these nodes however showed them to be markedly disorganized. If these nodes are accepted as having received adequate irradiation the percentage is then 90 per cent. There was 1 death in this group of animals. Dog No. 18 died on the forty-fourth postoperative day of pneumonia which was thought not to be related to gold therapy. This dog's bone marrow was normal. All animals had well healed bronchial stumps at the time of autopsy.

In the second experiment 12 dogs were subjected to pneumonectomy with the chest still open. 45 to 51 mc (in 10 cc of solution) of radioactive silver coated gold colloid were injected into the mediastinum under direct vision. All of these animals showed histologic destruction of the superior mediastinal group of nodes. Radioactivity was also high as determined by the Texas well counter (Table 2). However 3 dogs (25 per cent) showed evidence of a bronchial stump fistula.

In the third experiment 10 dogs were subjected to lobectomy. At the time of operation 35 to 50 mc of the isotope were placed in the pleural space.

*Table 1 Group 1 Dogs Receiving Intrapleural Gold Colloid Following Pneumonectomy**

NUMBER	DOSAGE	β SUPERIOR ANTERIOR MEDIASTINAL	β SUPERIOR MEDIASTINAL LEFT	β SUPERIOR MEDIASTINAL RIGHT
26	44.4 mc	28,546	5,855	13,240
27	44.4 mc	199,254	181,032	315,675
14	50.0 mc	6,211	96	85
12	50.9 mc	88,632	3,451	2,096
8	52.0 mc	198,077	9,869	48,735
9	52.0 mc	83,220	52,440	96,777
10	52.0 mc	34,910	16,783	Not counted
18	75.0 mc	150,314	Count too near background count	Count too near background count
19	75.0 mc	365,489	Count too near background count	Count too near background count
28	150.0 mc	94,620	86,549	181,716

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INTRA OSSEOUS VENOCRAPIH *

Its Value to the Surgeon

ROBERT SCHOBINGER VON SCHOWINCEN AND FRANZ P. FESSMANN

By means of intra-osseous venography (IOV) certain venous pathways which hitherto partially or completely escaped visualization may be demonstrated. The governing principle of this new technique is the injection of contrast medium directly into the medullary cavity of bony structures. The diffusion of the so introduced contrast substance is on the one hand influenced by the hydrostatic pressure within the medullary cavity and on the other by the hemodynamic force within the systemic venous vascular bed. The contents of the medullary cavity act as a poorly compressible column enclosed in rigid walls. The injected contrast substance therefore diffuses under normal conditions over only a moderate distance within the medullary canal and chooses the path of least resistance. This results in the prompt escape of contrast medium from the bone into the systemic venous circulation which rapidly transports the substance in centripetal direction.

The following remarks are based upon our personal experience with IOV in over 300 patients.

METHOD

Local anesthesia is entirely sufficient. Adequate sedation is obtained with thorazine (25 mg), demerol (75 to 100 mg) and seconal (0.1 gm). The former drug is administered 2½ hr and ½ hr; the latter two 1 hr prior to the procedure. The necessary precautions against idiosyncrasies to the local anesthetic agent or the contrast medium must be taken. A 16 gauge Rosenthal bone marrow aspiration needle (BD #160 LNR) or the longer 16 gauge Lundy Irving needle (BD #161 LNRC) in case of trochanteric injection is introduced into the medullary cavity of the selected bone. The correct positioning of the needle is verified by aspiration of bone marrow which may be saved for bone marrow smears. The contrast medium used is 50 per cent Hypaque. After introduction of 2 cc of contrast substance a scout film is taken verifying the correct position of the needle as well as of the patient. Then 10 to 15 cc of Hypaque are injected under constant pressure except for the demonstration of the vertebral plexus where forceful and rapid injection is required. Roentgenograms are taken during the injection of the last 2 to 3 cc of contrast medium. Straining motion and forceful breathing by the patient should be avoided since increase of the intrathoracic or abdominal pressure (Valsalva maneuver) may result in poor or absent filling of certain veins. The demonstration of the vertebral venous plexus is best accomplished by compressing the inferior vena cava with some radiolucent but firm material. This temporary obstruction results in a shunt of venous blood over the vertebral plexus. For more detailed information concerning method see Table I.

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The histologic changes in the mediastinal lymph nodes of all 3 groups can be considered together. There was total necrosis of the nodes and the immediate surrounding tissues in those instances in which the radiological effect was the greatest. In the less severe cases the nodal architecture was partially preserved, but with a complete loss of lymphocytes, plasma cells and reticulum cells were prominent survivors in these nodes. Erythrophagocytosis was noted in lymph nodes with high counts in animals that were sacrificed early. It is notable that the vascular damage frequently assumed a form which strikingly resembles fibrinoid necrosis.

DISCUSSION

One can conclude from these experiments that the lymphatics of the parietal and mediastinal pleura in dogs drain into the mediastinal lymph nodes bilaterally. These nodes may be destroyed by the intrapleural or intramediastinal injection of radioactive silver coated gold colloid. It is felt that if these observations prove to be valid in the human, a prophylactic type of mediastinal dissection may be added to pneumonectomy in the treatment of patients with resectable bronchogenic carcinoma. Although we do not think it likely that lymph nodes replaced by tumor would pick up sufficient radioactive material to destroy them, it is not unreasonable to believe that small tumor emboli in these nodes could be destroyed by this method. We do not advocate the mediastinal injection of this drug in humans because of the high incidence of bronchial stump dehiscence in dogs.

Six patients have been treated with radioactive silver coated gold following pneumonectomy for bronchogenic carcinoma at the Thayer Veterans Administration Hospital. No ill effects have been observed from this therapy. Dosage has ranged from 75 to 150 mc. Four of these 6 patients are alive 4 to 26 mo following therapy without evidence of recurrent tumor.

SUMMARY

1. Radioactive silver coated gold colloid consistently produces a radiologic effect upon the mediastinal lymph nodes of dogs following intrapleural injection and following injection into the mediastinum.

2. No harmful effects have been noted following intrapleural administration. The incidence of bronchial stump dehiscence following the intramediastinal injection of the colloid in pneumonectomized dogs was 25 per cent.

3. Currently we are treating humans with the gold colloid following resective surgery for bronchogenic carcinoma. We do not advocate the intramediastinal injection of the drug because of the high incidence of stump dehiscence in dogs.

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Malignant lesions of the right lung especially those located in the upper lobe were in some instances found to produce a complete block of the azygos vein as evidenced by its lack of filling. Occasionally only partial obstruction or mere compression of the azygos vein was observed. All right sided inoperable pulmonary neoplasms or cancers did present a complete block of the azygos vein. In the presence of some operable lesions however a lack of filling or compression of the azygos vein was as verified during surgery found to result from the huge size of the neoplasm itself or from enlarged azygos nodes. Benign or malignant lesions of the left lung whether operable or inoperable did not appear to obstruct the azygos vein. In one instance of an inoperable neoplasm of the left upper lobe involving the aortic arch a mere displacement of a normal calibered azygos vein to the right was observed. Hence the value of IOV in evaluating the operability of pulmonary lesions is at the present time at least difficult to assess. In other instances a previously blocked azygos vein may again be visualized after radiation therapy.

Esophageal neoplasms of all intrathoracic levels did in a limited series of 6 cases not appear to affect the course of the azygos vein. Mediastinal tumors associated with a superior vena cava syndrome caused obstruction of the azygos vein with deviation of the flow of contrast medium into distal veins or collateral channels such as the vertebral plexus lateral thoracic and ascending lumbar veins.

Pelvic masses be they the primary lesion itself extension of the primary neoplasm or metastatic nodes may lead either to complete obstruction deviation or compression of the external and internal iliac veins. In case of pelvic venous obstruction a certain amount of collateral circulation over the presacral iliac sciatic obturator and gluteal veins may be demonstrated. This is frequently associated with an increased filling of the femoral vein and occasionally with marrow stasis on the involved side. The origin and the type of malignancy appears to be less contributory to the degree of intra pelvic venous obstruction than the extent and location of the lesion. Chronic inflammatory changes other than pelvic phlebitis (we had no experience with this latter condition) and perhaps fibrotic changes have the tendency to distort the course of the veins and give their walls a ragged appearance frequently without obstruction. On the other hand complete lack of filling of the corresponding internal iliac vein was observed in 1 case of acute pelvic inflammatory disease and in 1 instance of tubal pregnancy (venous thrombosis).

Studies of the external and especially of the internal vertebral plexus of the dorsolumbar spine have been carried out in presence of metastatic traumatic and degenerative vertebral lesions as well as in a few instances of disc protrusion. Interruption of the flow of contrast substance within the internal vertebral plexus was present in all our cases with metastatic neoplastic vertebral involvement even in early lesions with normal myelograms. Occasionally there was also blockage of the ascending lumbar vein with distal deviation of the flow of contrast medium. Vertebral body disorders due to trauma or hypertrophic changes did not alter the pattern of the internal vertebral plexus on lateral views. Degeneration of the intervertebral disc may produce a variable degree of unilateral or bilateral blockage or none at all depending upon the severity of the protrusion. These changes are much better

Table 1

POSITION AND SITE OF INJECTION	AREA VISUALIZED
Antero posterior view Tenth rib in right mid axillary line Patient supine Contrast substance passes through the corresponding intercostal vein	Ceatear azygos vein
Lateral view Spinous process of D ₁₁ or D ₁₂ Patient in straight lateral position	Hemazygos vein
Injection similar to above but on the left	Collateral circulation in presence of portal hypertension
Midsternal line level of 4th rib Patient supine Syringe connected to needle by plastic tubing (bilateral visualization)	Internal mammary veins
Fourth or 5th rib of corresponding side anterior axillary line Patient supine (unilateral visualization)	
Acromial process head of humerus distal clavicle mid thoracic ribs in mid axillary line In the latter instance the substance flows over the lateral thoracic vein Patient supine	Subclavian vein
Spinous process C ₂ Patient upright Postero anterior view of cervical spine	Vertebral veins
Mid axillary line of corresponding rib	Intercostal veins
Dorsal or lumbar spinous process of involved vertebra or of vertebra immediately below Compression of inferior vena cava Patient in straight lateral or prone position Latter provides much more detail	Internal and external vertebral plexi in area over 2-4 vertebrae corresponding to site of injection
Protuberances of peripheral bone	Deep and sometimes superficial venous circulation of corresponding extremity
Distal protuberances or distal end of shaft of long bones with additional needle in proximal end of bone in order to decrease the intramedullary pressure by escape of bone marrow through the proximal needle and allow ascension of contrast substance within shaft	Medullary cavity of long bones
Line crest greater trochanter pubic bone Patient supine Simultaneous bilateral injection provided best results The more medial the site of injection the more veins of the true pelvis are demonstrated	Pelvic system
Angle of mandible	Medullary cavity of mandible Metryoid plexus on corresponding side

RESULTS

Review of examinations carried out in patients with benign or malignant lesions involving the lungs mediastinum esophagus pelvis breast spine long bones ribs and mandible and with portal hypertension varicose veins is well as prior and after radical neck dissections reveal that some observations are by necessity provisional while others are relevant of a definite pattern.

Malignant lesions of the right lung especially those located in the upper lobe were in some instances found to produce a complete block of the azygos vein as evidenced by its lack of filling. Occasionally only partial obstruction or mere compression of the azygos vein was observed. All right-sided inoperable pulmonary neoplasms or cancers did present a complete block of the azygos vein. In the presence of some operable lesions however, a lack of filling or compression of the azygos vein was, as verified during surgery, found to result from the huge size of the neoplasm itself or from enlarged azygos nodes. Benign or malignant lesions of the left lung whether operable or inoperable did not appear to obstruct the azygos vein. In one instance of an inoperable neoplasm of the left upper lobe involving the aortic arch a mere displacement of a normal calibered azygos vein to the right was observed. Hence the value of IOV in evaluating the operability of pulmonary lesions is at the present time at least difficult to assess. In other instances a previously blocked azygos vein may again be visualized after radiation therapy.

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Studies of the external and especially of the internal vertebral plexus of the dorsolumbar spine have been carried out in presence of metastatic traumatic and degenerative vertebral lesions as well as in a few instances of disc protrusion. Interruption of the flow of contrast substance within the internal vertebral plexus was present in all our cases with metastatic neoplastic vertebral involvement even in early lesions with normal myelograms. Occasionally there was also blockage of the ascending lumbar vein with distal deviation of the flow of contrast medium. Vertebral body disorders due to trauma or hypertrophic changes did not alter the pattern of the internal vertebral plexus on lateral views. Degeneration of the intervertebral disc may produce a variable degree of unilateral or bilateral blockage or none at all depending upon the severity of the protrusion. These changes are much better

appreciated on postero interior views since on straight lateral views the ascending lumbar veins may overlap the path of the internal vertebral plexus in the lumbar region. By temporarily compressing the inferior vena cava with the patient in prone position myelogram like patterns of the internal vertebral plexus may be obtained and thus without introduction of contrast medium into the spinal canal itself.

Presently we are evaluating the flow through the vertebral veins before and after neck dissections in an effort to correlate the postoperative symptomatology with the degree of cephalic venous stasis especially in cases of bilateral neck dissection. Preliminary information points to a poor, cephalo-cervical venous drainage in cases with increased postoperative cephalic symptomatology as evidenced by the demonstration of narrow cervical veins in these patients as compared with normal or asymptomatic individuals.

By means of venography of the internal mammary veins it is anticipated that better preoperative evaluation of the spread of breast carcinoma will be possible. Up to the present time, 10 unselected cases with benign and malignant breast lesions have been screened with internal mammary venography. In all instances where either deviation or obstruction of the internal mammary vein was noted a biopsy of that area was obtained. Prominent retrosternal adipose tissue, postirradiation pleural thickening, abnormally narrow internal mammary vein segments and metastatic node involvement of variable degrees were found to be the cause of such venous alterations. What appears established is that this type of examination may guide the surgeon to biopsy the correct site of possible metastatic spread and lead to a more precise therapeutic approach, be it by surgery or irradiation. Furthermore the demonstration of possible retrosternal lymphatic spread in clinically cured patients may not only lead to a better evaluation of follow up results but also allow earlier institution of secondary therapy.

The demonstration of an increased collateral venous circulation involving mainly the intercostal veins and vertebral plexus in patients with portal hypertension and with or without radiologically demonstrable esophageal varices, may prove to be of definite diagnostic and therapeutic value to the surgeon. It is conceivable that demonstration of such collateral systemic venous channels in patients with upper gastrointestinal hemorrhage of unknown origin would point toward the probability of bleeding varices rather than of a bleeding ulcer. However this promising avenue which we recently entered must be more thoroughly exploited before definite conclusions can be reached, one of them being that the demonstration of such a collateral circulation in an early cirrhotic but otherwise asymptomatic patient could allow shunt procedures to be performed at a much earlier and therefore favorable time.

Intraosseous venography is advantageously used in the demonstration of the deep venous circulation of the leg since the flow of the introduced contrast substance is into the deep systemic veins. Better evaluation of deep varicose veins and associated disorders such as the localization of a phlebotic obstruction may be carried out without contraindication since the contrast substance is not introduced directly into the venous circulation. The superficial veins may or may not be visualized by this method but failure to visualize the deep veins speaks in itself unquestionably for a block.

The uniformity of intramedullary diffusion of contrast medium may be

altered by certain disorders. The present study was mainly concerned with the detection of early metastatic lesions in the ribs. Such neoplastic deposits were found to obstruct the intramedullary propagation of contrast medium with concomitant formation of collateral channels in the area of the lesion. Principally, the same approach may be utilized in studying the intramedullary circulatory pattern in the presence of benign or malignant conditions in other locations. Our present investigations in this regard comprise studies concerning the local extent of tumors of the jaw, localization of tumors of peripheral bones and circulation in fractures.

SUMMARY

The authors have submitted their observations as obtained by means of intra-osseous venography. The demonstration of certain hitherto hidden venous pathways such as the study of the internal mammary, azygos and pelvic veins as well as vertebral plexi proved to be of definite value to the surgeon. The great potentialities, the simplicity of technique, and the relative safety of intra-osseous venography certainly invite further investigative work.

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THE CIRCULATION OF BONE WITH PARTICULAR REFERENCE TO THE MEDULLA AND MEDULLOGRAPHY*

Intraosseous Phlebography

RUTHERFORD S GILFILLAN, NICHOLAS L. PETRAKIS
AND HOWARD L. STEINBACH

The circulation in the hemopoietic bone marrow is a dynamic one in direct communication with the extraosseous systemic circulation as manifested by pressure measurements,¹ clearance of isotopes,² and opaque media. Study of the medullary pressure reveals a pulse wave as well as respiratory fluctuation and response to vasoconstrictors such as adrenalin. The Valsalva maneuver (forced expiration against a closed glottis) is followed by a prompt rise

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Fig. 1 Vinylite injection of the arterial system of a 5 mo fetus A Nutrient artery of tibia B Sinusoids filled in upper diaphysis and metaphysis of tibia C Small branches of geniculate artery forming metaphyseal arteries D Nutrient artery vein and sinusoids outlining distal diaphysis and metaphysis of femur

in intramedullary pressure and the Mueller maneuver (forced inspiration against a closed glottis) produces a drop in pressure. Injection of I^{131} into the marrow cavity is followed by a clearance rate approximately similar to that of muscle. Injection of contrast media (Hypaque) into the ends of long bones shows its removal to be more rapid than that from muscle or other soft tissue. The ease and rapidity with which large quantities of fluid can be introduced into the marrow demonstrates not only the capacity of the venous outflow tract but also suggests that the introduction of opaque media in a similar fashion might provide a radiographic means of demonstrating the venous drainage and visualization of the veins of the surrounding soft tissues.

Knowledge concerning the morphology of the circulation of bone in man and animal has been derived from the analysis of the work of Doan³, Drinker⁴ and Trueta⁵ and by the careful inspection of actual injections of vinylite followed by corrosion in fetuses and stillborn infants (Fig. 1). Direct injection of amido trizoate sodium (Hypaque) 50 per cent into the medullary canal of bone affords further data concerning the flow within the bone and its venous drainage.

The arterial supply of cortical or compact bone is derived from the numerous vessels ramifying in the periosteum and connecting with the longitudinal Haversian canals through the horizontal canals of Volkmann. This supply also is in continuity with the vessels of the medullary canal. The cancellous or spongy bone is supplied by less numerous but larger vessels; the numerous openings for which are readily visible near the articular surfaces at the ends of long bones or in relation to musculo-tendinous attach-

Fig 2 Foramina for transmission of vessels in the condyles of the femur (left) and in the anterior tibia just below the patella (right)



ments (Fig 2) Some of these openings transmit arteries but the majority of them form a path for emergence of veins. A third supply is that of the nutrient artery and vein or veins which are usually seen entering the shaft of a long bone and pursuing an oblique course through the cortex to divide on reaching the medullary canal proximally and distally to anastomose with the preceding described sources of blood flow. These vessels during development might be considered as the blood supply of the diaphysis and end vessels to the metaphysis. The nutrient artery and vein become smaller with increasing age and development because of the decreased blood supply necessary in the shift as a result of replacement of the active hemopoietic bone marrow with the less vascular fatty marrow. The sinusoids for the most part are flat ovoid spaces connected at two or three points to adjoining spaces or vascular channels. The sinusoids of the epiphysis and the diaphysis are in direct continuity following the cessation of growth and the disappearance of the epiphyseal plate.

METHOD

The injections in this series were carried out with a standard Turkel bone marrow needle with trephine under either local or general anesthetic. The injection of the contrast media sometimes proved to be extremely painful because of the high pressures exerted in the initial phases of the injection. On 2 occasions the appearance of pain was correlated with the pressure exerted by attaching a flask of saline to the needle and elevating it above the injection site at the malleolus of the tibia. Discomfort was not noted until the flask was elevated 290 cm above the tibia on the first occasion and 300 cm on the second occasion. Because of the pain a short acting general anesthetic was commonly used.

The needle was inserted into the distal end of a long bone or directly into a flat bone with a rotary movement. One to 1 cc of bone marrow was aspirated followed by the injection of 10 to 20 cc of an aqueous contrast media. Hypaque 50 per cent was the contrast media used in all instances in this study. The first roentgenogram was usually exposed immediately after the completion of the injection. Additional roentgenograms were obtained at suitable intervals which varied depending upon the purpose of the examination. The use of a plastic tube connecting the syringe to the bone marrow needle was of value.



Fig. 1 Vinylic injection of the arterial system of a 1.5 mo fetus A Nutrient artery of tibia B Sinusoids filled in upper diaphysis and metaphysis of tibia C Small branches of geniculate artery forming metaphyseal arteries D Nutrient artery vein and sinusoids outlining distal diaphysis and metaphysis of femur

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DISCUSSION

The contrast material injected into cancellous bone presents a homogeneous poorly delineated shadow in the roentgenogram which persists after the major portion of the medulla has drained to the systemic extra osseous veins. The majority of the injection mass passes rapidly into small interlocking channels which rapidly converge to form larger trunks to emerge from the bone and fill the deep and superficial soft tissue veins (Fig 3). Injection into long bones is usually followed by filling of the medullary vein for approximately one third of the length of the shaft. The so called medullary vein is a tortuous irregular channel sometimes presenting the appearance of a group of communicating sinusoids. The medullary vein may be more prominent with the presence of a tumor probably due to increased flow in

Fig 7. Nodular vasculitis of both legs. Injections into the tibial and talarus of both legs with filling of deep and superficial veins of leg and thigh.



Fig 8. Deep and superficial thrombophlebitis. Injection into medial condyle of the femur. Femoral vein is obstructed and the thrombosed saphenous vein is not visualized. Marked edema was present.



Fig 9. Multiple myeloma. Injection into the ilium with visualization of the superior gluteal and hypogastric vein.



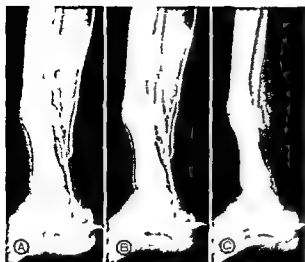


Fig 3 Old non union fracture of distal one third of tibia and fibula. Injection into calcaneus 20 cc. 50% Hypaque. The tortuosity and irregularity in the region of the old fracture are due to the venous damage at the time of fracture. A At completion of injection. B At 15 seconds. C At 45 seconds.

Fig 4 Osteogenic sarcoma shaft of left femur. Injection of 50% Hypaque into lateral condyle. Medullary vein is large, irregular and tortuous. The media passes readily to the soft tissue tumors.



Fig 5 Cavernous venous hemangioma of vastus medialis. This residual tumor demonstrated following surgical removal. Injection site medial condyle of femur.



Fig 6 Varicose veins of the left leg. Injection of 50% Hypaque into tibial malleolus. The contrast media spread across the epiphyseal line outlining the deep and superficial circulation. There is a perforating vein visible at mid shaft communicating with a vein posterior to the saphenous vein.

ARIAL METASTASIS OF TUMORS IN THE LUNG AN EXPERIMENTAL STUDY IN MICE*

LOUI D. NORMAN AND ROBERT D. McBROOM

The possibility of arial metastasis of tumors within the lung has been largely overlooked in clinical and experimental cancer investigations. Isolated examples of this route of tumor spread in man have been reported but these cases have not been conclusive enough to establish it definitely as a mechanism of tumor dissemination.

Experimental evidence of the role of the bronchi in tumor spread was reported by Furth¹ in 1946. tumor cell suspensions placed into the nares of mice resulted in pulmonary tumors in a significant number of animals. The tumors used were a mouse lung carcinoma and leukemias.

The present investigation was initiated in order to study the incidence and morphology of lung tumors in the mouse resulting from the injection of tumor cell suspensions directly into the trachea as well as into sites adjacent to the upper respiratory passages.

METHODS

C₃H mice of mixed sex were used in all of the experiments. these animals were supplied with Purina mouse food and water *ad libitum*. Tumors used were a mammary adenocarcinoma (C₃HBA) a hepatoma (98/15) and a fibrosarcoma (HL 8971).²

Tumor cells were suspended in normal saline in a concentration range from 10 to 50 per cent. Mice were divided into groups for injection of the tumor cells as follows: intratracheal (mammary tumor) 10 mice; intratracheal (hepatoma) 9 mice; intratracheal (fibrosarcoma) 12 mice; submucosa of mouth (mammary tumor) 13 mice; and submucosa of nares (mammary tumor) 6 mice. With the mice anesthetized by intraperitoneal pentobarbital the intratracheal injections were made by insertion of a dull 20 gauge needle through the mouth and larynx into the lumen of the trachea. Placement of the needle in the trachea was checked by inspection through an incision anterior to the trachea. The amount of cell suspension injected ranged from 0.05 to 0.1 ml. but most of the mice received 0.05 ml. Some of the mice which had submucosal injections of tumor cells in the mouth were anesthetized and had incisions made over the tumor once or twice during its development. Except for the few mice that died immediately after injection the interval between instillation of the tumor cells and death or sacrifice of the animal was as follows: intratracheal (mammary tumor) 22 to 33 days; intratracheal (hepatoma) 31 to 91 days; intratracheal (fibrosarcoma) 14 to 32 days; submucosa of mouth 10 to 18 days; and submucosa of nares 12 to 17 days. At autopsy the lungs from each mouse were examined for gross tumors, fixed in 10 per cent formalin, imbedded in paraffin and multiple sections stained with hematoxylin and eosin for microscopic examination.

RESULTS

Intratracheal administration. Of the 10 mice injected with the mammary tumor 3 died immediately after receiving the cell suspension. Seven mice

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the region of the tumor' (Fig 4) Other tumors such as hemangioma of the adult venous type are easily visualized (Fig 5) Injection of the epiphyseal region in the adult is followed by spread of the contrast media across the epiphyseal line (Fig 6) This does not occur during the active growing period

Injections into the tibia, fibula, femur, and calcaneus fill the deep and superficial veins making possible the visualization of the perforating veins For this procedure the patient should be tilted with the legs dependent (Fig 7) This is of value in an edematous and ulcerated extremity or in an extremity where thrombosis of superficial veins is feared (Fig 8) The veins of the pelvis may be visualized by injection of greater trochanter of the femur, the pubis or the iliac crest (Fig 9) The azygos system can be visualized via the vertebral body or spinous processes

SUMMARY

This method of examination is useful in the study of veins in relation to

- 1 Thrombosis and varicosis
- 2 The delineation of tumors and vascular alterations produced by them
- 3 The study of the anatomic and pathologic status of bone and hematopoiesis

Its disadvantage is that of requiring anesthesia Slower injection rates may obviate the necessity for anesthesia

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produced in the lungs were identical morphologically to the tumors used to prepare the cell suspensions

The reasons for the much lower rate of successful pulmonary implantation with the hepatoma and fibrosarcoma are not clear and this phase of the problem needs further study. The only explanation offered is that in the case of the hepatoma the percentage of ticks subcutaneously is considerably less than with the other tumors used; that tumor is also much slower growing than the other neoplasms.

The few tumors that occurred in the lungs of the mice that had injections of tumor cells into the submucosa of the mouth were evidence that sloughing tumor cells can be disseminated through the air passages to produce metastatic lesions. The protrusion of the tumors into the mouth with ulceration of the overlying mucosa gave support to that concept. No proof can be given that the lung tumors did not occur by lymphatic dissemination from the primary transplant and one of the animals especially raises the possibility of such spread since the tumor developed in the mediastinal pleural and subpleural regions. However, the absence of tumor in all lymph nodes examined is evidence against a lymphatic type of metastasis. It was planned to include control animals with injections subcutaneously in the cheek; however, it became apparent that with growth of the tumor the soft tissue of that general area was involved extensively without regard to the exact point of injection. Studies are planned using injections subcutaneously in the neck to rule out the possibility of lymphatic metastasis to the lung. This experiment was made difficult by the fact that survival time of the mouse after development of a tumor in the mouth region is less than the period necessary to develop tumors of even very small size following cell dissemination through air passages.

SUMMARY

Metastatic lung tumors have been produced in the C_3H mouse following intratracheal instillation of cell suspensions from 3 tumors: a mammary adenocarcinoma (C_3HBA), a hepatoma (98/15) and a fibrosarcoma (HL 8971). The mammary tumor developed in the lung much more readily than did the other 2 neoplasms.

Four tumors developed in the lung after the submucosal transplantation of the mammary tumor in the mouth. It is believed that this represents development of tumor from the sloughing of tumor cells and their dissemination through the air passages; but the possibility of lymphatic spread can not be absolutely ruled out as the responsible mechanism.

It is concluded that these experiments support the concept of aerial dissemination of tumors.

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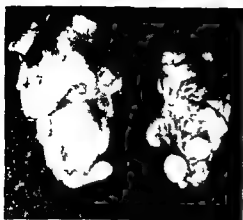


Fig 1 Gross tumor nodules in the lungs of a mouse in which a suspension of the mammary tumor cells had been injected intratracheally

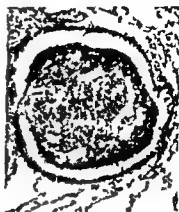


Fig 2 Tumor in bronchiole of the lung shown in Figure 1

developed multiple pulmonary tumors, the gross appearance of which is illustrated in Figure 1. The size of these tumors varied roughly with the length of the post injection period.

Injection of the hepatoma cell suspension into the trachea resulted in 1 tumor of microscopic size. 4 of these animals did not survive the initial inoculation of the tumor cells. Two of the mice injected with the fibrosarcoma died immediately. 1 of the remaining 10 mice developed gross tumors.

Microscopically these tumors could be seen filling bronchi and bronchioles (Fig 2) and extending out into adjacent lung tissue. In some areas masses of tumor not related to visible bronchi were noted. Cell detail of the tumors did not differ from that seen in these neoplasms in subcutaneous type of implants.

Submucosal injection (mouth) Only the mammary tumor was used for these studies. Of the 43 mice inoculated with the tumor cells 9 died immediately. Of the remaining animals 4 developed pulmonary tumors, all of them discernible only by microscopic observation. Two of the lung tumors occurred in animals whose mouth tumors were incised during their development. In 1 animal the tumor was subpleural and pleural in location and tumor was also present in the mediastinum. All of the tumors were identical to the primary tumors in the soft tissues of the mouth.

Submucosal injection (nares) None of these mice died after injection and none developed tumors.

DISCUSSION

The tumors which developed in the lungs following the intratracheal injections were very definitely growing out of bronchi. It was felt that control animals injected with material other than tumor cell suspensions were not necessary for the following reasons: spontaneous tumors have not been noted in these mice at the age range used, and both spontaneous pulmonary tumors and induced non-neoplastic proliferative disease can easily be separated histologically from these tumors. There was no doubt that the tumors

Table 1. Challenge Dose of 20 Million Tumor Cells

NO MICE	TREATMENT	NO DIED	MEAN SURVIVAL (DAYS)
10	Antiserum	10	118
10	Controls	10	108
10	Lymphocytes	10	112
10	Controls	10	106

bled and the serum collected. At the same time the spleen was removed and a washed suspension of spleen cells in balanced Ringer's solution was prepared.

The antiserum and lymphocyte suspension were stored at about 2° C until injected. Penicillin and streptomycin in small quantities were added to each solution. The longest each solution was stored prior to injection was 4 days.

Susceptible mice were injected with viable tumor cells, the size of the challenge dose being computed by means of hemocytometer counts of single cell suspensions of the tumor. Treatment was instituted with either antiserum or lymphocyte suspension within 12 to 18 hr.

In the first group (Table 1) all mice were challenged with a large dose of tumor cells (20 000 000). They were divided into 4 groups. One group was selected to be treated with antiserum and another to receive the lymphocyte suspension. The remaining 2 groups served as controls. Those receiving antiserum were injected daily with 0.1 cc subcutaneously for 6 days. The group given the lymphocyte suspension received 0.2 cc of the suspension daily for 6 days.

In the second group (Table 2) the challenge dose of tumor cells varied from 10 000 to 1 000 000. Antiserum alone was used for treatment in this group. The dosage schedule was the same as for the first group. Each of the 3 subgroups had control counterparts.

RESULTS

It may be noted in Table 1 that when a large dose of tumor cells was injected all animals in both the control and treated groups succumbed to the disease but there was a definite increase in survival time in the treated groups.

Table 2 demonstrates the complete protection afforded by the antiserum when the challenge dose of tumor cells is minimal.

Table 2

NO MICE	TUMOR CELL DOSE	TREATMENT	NO DIED
12	10 000	Antiserum	0
12	10 000	Controls	10
12	100 000	Antiserum	0
12	100 000	Controls	12
12	1 000 000	Antiserum	2
12	1 000 000	Controls	12

TREATMENT OF EXPERIMENTAL LYMPHOID TUMOR WITH HETEROLOGOUS ANTISERUM AND IMMUNE LYMPHOCYTES*

JAMES T. GRACE, FRANK GOLLAN AND ROBERT I. CARLSON

In recent years there has been a considerable revival of interest in the immunologic approach to cancer research.

Early reports of the use of antiserum prepared against malignant tissue in the treatment of experimental cancer showed equivocal results. Possible explanations for these failures have been discussed by Pressman.¹

Numerous other studies have demonstrated that tumor specific antibodies can be produced in the homologous or heterologous animal by injection of either whole tumor cells or certain fractions thereof. Furthermore, the cytotoxicity of such antibodies for the specific tumor cell has been amply shown. Incubation of viable tumor cells with such antiserum prior to injection into the susceptible animal prevented the production of the disease.

More recently, Gorer and Amos² have shown that mice could be afforded considerable protection against inoculation with a strain susceptible leukemia by pre-treatment with iso-immune serum.

This study was undertaken to assess the value of hyperimmune heterologous serum on the course of a mouse leukemia when treatment with the antiserum was begun after inoculation with the disease.

No attempts were made to remove antibodies from the antiserum which may have been formed against normal tissue components by absorption or neutralization techniques.

It was further desired to note also the effect of the use of cellular elements from the heterologous animal. For this purpose a washed suspension of spleen cells was employed.

METHOD

The malignant disease used in this study was the P1534 leukemia carried in the DBA/2 strain of inbred mice.³ Within 5 to 7 days after inoculation subcutaneously with this type tumor cells there appeared a palpable tumor at the site of injection. This rapidly progressed to a typical lymphatic leukemia which was fatal in 10 to 11 days when a standard tumor cell dose inoculum was used.

The standard laboratory white rabbit was employed for the production of antiserum and the antigen consisted of a suspension of spleen cells from mice moribund with the disease.

The rabbits were injected initially intraperitoneally with 2 cc. of an emulsion consisting of equal parts of antigen and Freund's adjuvant. The amount of splenic tissue injected was equal to approximately 100 mg. of wet weight. Subsequently, 2 additional injections of such an emulsion were made at 2 wk. intervals, these being given subcutaneously at multiple injection sites. Two weeks following the last injection a final boost of antigen in saline was given subcutaneously. Ten days after this the animals were

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considered the result of an autotransplantation of tumor cells detached from the primary growth it was thought that the host might show a corresponding increase in susceptibility toward a reinoculation of the same tumor concomitant with an increasing size of the primary

METHOD

To test this hypothesis a series of experiments was conducted in the following way. Z(C₃H) black cross hybrid mice (ZBC) were used as recipient hosts. The tumor employed was a transplantable mammary adenocarcinoma originally arising in a Z(C₃H) breeder mouse which had been maintained in this laboratory for 53 successive transfers into mice of the Z(C₃H) strain F₁ hybrids and ZBC black-cross hybrids without losing its original genetic characteristics since it does not grow in any strain other than those listed.

Mice were first inoculated with this tumor into the subcutaneous tissue of the tail by injecting 0.05 cc. of a tumor cell suspension prepared as an 8 per cent concentration in saline. At 12, 17 and 22 days after transplantation groups of mice growing caudal tumors were submitted to amputation of their tails by clipping them off near the root of the tail with a pair of hot sterilized scissors. The removal of the tail was followed in each mouse by the subcutaneous inoculation into the right groin of 0.25 cc. of a second tumor suspension prepared as a 5 per cent concentration. The tumor used for the second inoculum was taken from the same group of donors used for the original tail transplant.

Each group of experimental animals had its own controls consisting of mice receiving the second transplant after clipping off their normal tails. After the groin implant mice were inspected once every other day for the appearance of subcutaneous tumors at the site of inoculation and the progressive incidence of takes determined in the various groups.

RESULTS

Tables 1 and 2 show the results obtained in 2 similar experiments. As can be seen (Table 1) the second inoculation made at 12 days after the tail transplant produced new tumors earlier than in its controls although the final tumor incidence in both groups was almost the same (92 and 90 per

Table 1 Incidence of subcutaneous tumors obtained in mice reinoculated at different time intervals after a prior caudal transplant

TIME BETWEEN 1ST AND 2ND TRANSPLANT (DAYS)	GROUP	NUMBER OF MICE	PROGRESSIVE INCIDENCE OF SUBCUTANEOUS TUMORS AFTER THE SECOND IMPLANT (PER CENT)			
			DAY 12	16	20	24
12	Exper	37	3	76	92	92
	Controls	35	0	30	83	90
17	Exper	27	11	37	56	56
	Controls	30	13	37	73	93
22	Exper	28	0	0	7	7
	Controls	28	7	43	79	93

DISCUSSION

These studies demonstrate the ability of heterologous antiserum and spleen cell suspensions to influence the course of a malignant disease in the mouse. When a large tumor cell inoculation was followed by administration of relatively small quantities of these treatment materials all animals succumbed to the disease but showed a definite prolongation of survival time as compared to controls. However, when the tumor cell dose was markedly reduced and the antiserum doses remained the same it was possible to arrest the disease in practically all cases. This suggests that there is a direct relationship quantitatively between the number of tumor cells present and the amount of antiserum administered. If this simple relationship does exist it would appear that merely by increasing the number of antibodies more advanced disease could be controlled.

There are many problems which require further study. It is planned to evaluate the effect of larger doses of antiserum in the presence of more advanced disease. Additionally it is hoped that fractionization of serum proteins will yield a more concentrated solution of antibodies. Further study of the nature of the antigen and improved immunization procedures should produce higher antibody titers in the serum.

SUMMARY

Serum and lymphocytes from rabbits which were immunized with P1534 mouse leukemia favorably alter the course and mortality of the disease in susceptible mice when treatment is begun 12 to 18 hr after inoculation with viable tumor cells.

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IMMUNITY IN MICE TO STRAIN SPECIFIC TUMOR TRANSPLANTATION FOLLOWING GROWTH AND REMOVAL OF THE SAME TUMOR*

CARLOS MARTINEZ, J. BRADLEY AUST AND JOHN J. BITTNER

It has previously been established that the incidence of lung metastases in mice growing a transplanted tumor is related to the size of the primary, i.e. mice bearing large growths exhibited more metastases than those bearing smaller tumors (Wood *et al.*¹ Martinez *et al.*²). Since metastases may be

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experiments was able to induce immunity in mice reinoculated with Sarcoma 180 when tested 2 wks after the first transplant. His results were confirmed by one of us (J. J. B²) for Sarcoma 180 in some inbred stocks of mice but not in others. However he was unable to produce immunity when using a 7(C₃H) tumor retransplanted into mice of the same strain F₁ hybrids or 7BC hybrids 2 wks after crural inoculation. Our data are in agreement with these later results since using a similar tumor and the same type of mice no immunity was observed when the test was performed at 12 days. However at 17 and especially at 22 days immunity to the second inoculation was clearly established. To our knowledge this constitutes the first instance in which immunity could be demonstrated by using inbred stock mammary tumors.

The nature of the immunity observed in these experiments has not been clarified since preliminary attempts to transfer this immunity to normal mice using serum or splenic tissue of the resistant animals have been unsuccessful.

SUMMARY

Experiments in mice to test host susceptibility to a second inoculation of an inbred mammary tumor made at various intervals following a tail tumor transplant have shown the following results: a) if the second inoculation was made at 12 days after the crural transplant an acceleration in the take of the tumors in the experimental group as compared to the controls was observed; b) if the reinoculation was made at 17 and particularly at 22 days a high degree of resistance was observed. This has been interpreted as due to some form of immunity induced in the host by the continuous growth of the crural tumor.

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Table 2 Incidence of subcutaneous tumors in mice re inoculated at different time intervals after a prior caudal transplant

TIME BETWEEN 1ST AND 2ND TRANSPLANT (DAYS)	GROUP	NUMBER OF MICE	PROGRESSIVE INCIDENCE OF SUBCUTANEOUS TUMORS AFTER THE SECOND TRANSPLANT (PER CENT)			
			DAY 12	16	20	24
12	Exper	30	60	87	97	97
	Controls	30	90	100	92	100
17	Exper	29	0	0	10	19
	Controls	27	4	30	67	93
22	Exper	20	0	0	0	0
	Controls	23	10	26	61	87

cent respectively) The transplant made at 17 days after the first inoculation produced less tumors than in the control group (56 and 93 per cent respectively) the difference being statistically significant Finally in the group transplanted at 22 days only 7 per cent of the experimental group developed tumors as compared to 93 per cent of the controls

The results of a second experiment done under the same experimental conditions gave qualitatively the same results (Table 2)

It is interesting to point out that most animals in the 17 and 22 day groups had metastases in their lungs when sacrificed at the end of the experiment However there were no signs of malnutrition or cachexia in mice having metastases since the determination of the mean body weight in both experimental and control mice of the 22 day group did not reveal significant differences

DISCUSSION

The results of these experiments indicate that with the exception of the group re inoculated 12 days after the first transplant, which showed an acceleration in the takes the groups re inoculated at 17 and 22 days exhibited a marked resistance The acceleration observed might be interpreted as representing an enhanced susceptibility to tumor growth In this respect it is interesting to point out that the time at which the host seems to be hypersusceptible to autoimplantation is coincident with the time of initiation of the metastatic spread of the first tumor This is consistent with observations reported in previous studies (Martinez *et al*)

The basis for the resistance observed when mice were re inoculated at 17 and 22 days after the caudal transplant is not clear Most of the resistant animals exhibited lung metastases The question arose as to whether or not the presence of metastases in the lungs could interfere in some way with the take of the second tumor However in recent experiments to be published elsewhere the same phenomenon was obtained in animals in which metastases from the first tumor were prevented This would indicate that the presence or absence of metastases in the host does not play any role in the development of resistance to tumor reimplantation On the other hand it is conceivable that the continuous growth of the caudal tumor could have induced some form of immunity In fact Anderson¹ in similar

Table 1 Growth of Tumor Homografts in Mice

TUMOR	HOST	# ANIMALS	# TAKING	%
Z	Mature A	9	0	0
Z	Newborn A	17	9	53
Z	Newborn Cc	15	12	80
A	Newborn Z	8	8	100

Table 2 Excision and Replantation of Tumor Homografts Growing in the Newborn at the Time of Weaning

TUMOR	HOST	#	# REGROWING TUMOR
A	D ₈	8	0
Z	D ₈	6	0

Table 3 Histocompatibility Characteristics of Z Tumors Grown in A Mice

STRAIN	# MICE TREATED	# TAKING TUMOR
A	9	0
Z	9	9

lung metastasis at post mortem. Similar results were obtained using Z tumor into newborn Cc mice and A tumor into newborn Z mice with up to 100 per cent of the mice taking the tumor. In the earlier studies tumor suspension leaked from the site of needle puncture and may have accounted for the higher per cent of takes later when the needle site was coated with collodion. Groups of D₈ mice treated similarly with Z and A tumors in the newborn period took the tumors. At the time of weaning these tumors were removed, homogenized, a 10 per cent suspension prepared in saline and 5 ml reinjected into the same animal from which it was excised. Table 2 shows that the tumor failed to regrow in all instances indicating the absence of acquired tolerance at that time.

To test whether the Z tumor growing in an A mouse retained its normal histocompatibility characteristics the following experiment was carried out. The Z tumor which had grown in an A mouse for 1 mo was excised, a 5 per cent tumor suspension in saline prepared and 25 ml injected into the groins of 9 adult Z and 9 adult A mice. As is seen in Table 3 the tumor grew only in the Z mice indicating that it did retain its normal histocompatibility characteristics despite a period of growth in an A host.

DISCUSSION

These findings demonstrate that tumor homografts may be successfully transplanted between highly inbred strains of mice provided such a procedure is carried out in the first few hours of life. The mechanism responsible for this is not completely clear. Based on observations that some rats

TUMOR HOMOTRANSPLANTATION IN INBRED MICE DURING THE NEWBORN PERIOD*

J. BRADLEY AUST, CARLOS MARTINI, JOHN J. BITTNER AND ROBERT A. GOOD

It is an established fact that tumors arising spontaneously in one highly inbred strain of mice will not grow in an animal of another highly inbred strain. This rejection of tumor homografts in inbred strains of mice has been shown to be a function of the genetics of histocompatibility¹ and is identical in mechanism to homograft rejection.²

Recently Billingham^{3, 4} succeeded in producing a state of 'acquired tolerance' in mice characterized by their ability to accept homotransplants of skin from donors whose tissues had been implanted into the recipient during the embryonic period. Interest in this phenomenon had been stimulated by Owen, who discovered that dizygotic twin cattle may continue to circulate blood cells of 2 distinct genotypes (their own and their partner's). Reciprocal skin homografts will grow between such chimera, but they will not accept skin homografts from their mother, siblings, or other twin cattle.^{5, 6} The ability to produce acquired tolerance following introduction of living foreign cells in the mouse is optimal in the embryo and becomes gradually lost as birth approaches.⁴ Woodruff,⁶ studying the rat, found that varying degrees of acquired tolerance could be produced up to 2 wks after birth.

This report cites an attempt to produce 'acquired tolerance' in inbred strains of mice by implantation of tumor homografts in the neonatal period. The results indicate that such homografts will take and grow progressively. However, following removal of these tumors, the animals fail to exhibit acquired tolerance.

METHOD

Mice of the A/7 (C₃H) Ce and D₈ strains were used. A transplantable 7 mammary carcinoma of the 55th transplant generation and a transplantable A mammary carcinoma of the 1st transplant generation were the tumors employed. Each tumor grows only in members of its own specific strain. Fresh 5 per cent tumor concentrations in saline were prepared using a Potter-Elvehjem glass homogenizer. Mice of an homologous strain were injected subcutaneously under the nape of the neck before reaching 3 hr. of age. At 1 mo. of age the mice were weaned and the incidence of tumor was determined. Following this some of the tumors were excised, homogenized and reimplanted into their respective hosts.

RESULTS

Table 1 confirms that the 7 tumor used would not grow in mature A mice. However, when the tumor was implanted into newborn A mice over half of the mice grew the tumor progressively to death and exhibited

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TUMOR	HOST	#	# RETROWING TUMOR
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Z	D ₈	6	0

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STRAIN	# MICE TRIATED	# TAKING TUMOR
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Z	9	9

lung metastasis at post mortem. Similar results were obtained using Z tumor into newborn Ce mice and A tumor into newborn Z mice with up to 100 per cent of the mice taking the tumor. In the earlier studies tumor suspension leaked from the site of needle puncture and may have accounted for the higher per cent of takes later when the needle site was coated with collodion. Groups of D₈ mice treated similarly with Z and A tumors in the newborn period took the tumors. At the time of weaning these tumors were removed, homogenized in 10 per cent suspension prepared in saline and 5 ml reinjected into the same animal from which it was excised. Table 2 shows that the tumor failed to regrow in all instances, indicating the absence of acquired tolerance at that time.

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DISCUSSION

These findings demonstrate that tumor homografts may be successfully transplanted between highly inbred strains of mice provided such a procedure is carried out in the first few hours of life. The mechanism responsible for this is not completely clear. Based on observations that some rats

previously challenged during neonatal life with splenic tissue accepted one skin homograft but rejected subsequent grafts from the same animal. Woodruff postulated a so called critical period. He suggested that the homograft took during a period of partial tolerance and was resident in the host for a critical period long enough for the graft to adapt to the host and grow thereafter. Subsequent grafts at a time when tolerance had disappeared were rejected before such adaptation could occur. If this concept applies to our studies using tumor homografts, the original tumor when excised and reimplanted into the same host might be expected to grow again since it had already adapted to the host. However, this was not found to be true and all reimplantations of tumor failed.

The neonatal period represents a time when immunological capacity is poorly developed. At this time a tumor may take, establish itself, and possess sufficient growth potential to keep ahead of the developing immunological rejection forces. This would explain the failure of tumors to grow following excision and reimplantation in the same host, since at this latter reinoculation time the immunological forces of the host are more fully developed and cause rejection of the second graft. However, in these experiments when the original tumor was allowed to remain, metastasis occurred, throwing some doubt on the presence of immunological rejection forces at the time of metastasis.

Our results may be explained if the phenomenon of acquired tolerance is considered to be dependent on the presence of living foreign cells. In this circumstance the host allows growth of the tumor during a period when his immunological capacity is inadequate and the tumor by its growth maintains a state of acquired tolerance. Assuming metastasis to be new homo-transplants, their occurrence may be evidence of persisting acquired tolerance due to the presence of the primary tumor. When the tumor is removed before the occurrence of metastasis all living foreign tissue is removed; the animals are no longer tolerant and therefore fail to accept retransplantation of the same tumor.

SUMMARY

Tumor homografts will take and grow progressively between inbred strains of mice provided transplantation is performed within 3 hr after birth. These tumors retain their original histocompatibility characteristics despite growth in an homologous animal. It is proposed that living foreign tissue is required for the persistence of acquired tolerance.

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THE EXPERIMENTAL DESIGN OF STUDIES TO EVALUATE THE CHEMOTHERAPY OF SOLID TUMORS AS DEMONSTRATED BY THE USE OF TRITHYLINETHIOPHOSPHORAMIDE*

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In recent years there has been an ever increasing emphasis on the investigation of compounds with anti-tumor activity. The majority of clinical studies have been carried on in patients with leukemia and the malignant lymphomas.

A definite need exists for the systematic trial of these compounds in patients with solid tumors. Although the clinical effectiveness of most chemotherapeutic compounds upon solid tumors has been disappointing, tumor regression and suppression such as encountered in this study would indicate that future success will be obtained in this field. The importance of adding these new therapeutic techniques to our surgical armamentarium for the further control of malignancy is obvious.

A national program coordinating the exchange of information concerning synthesis of compounds, screening for anti-tumor activity, initial pharmacology studies, and clinical trials has been established. The Clinical Panel of the National Cancer Chemotherapy Center has made recommendations concerning the detection of compounds for human trial, criteria of evaluation, experimental design, and the number of patients which must be studied in order to obtain a valid evaluation of any one compound.

Selection of patients with measurable tumors is most important since after many discussions *the only acceptable criteria for chemotherapeutic effectiveness has been resolved to be the reduction in the size of the tumor by direct or indirect measurement.* Of course demonstrable extension of life is the ultimate measure of chemotherapeutic effectiveness, but even patients with rapidly growing tumors such as glioblastoma or gastric pancreatic and lung carcinoma could hardly be evaluated in less than two years. Such ancillary criteria as changes in the histological appearance of the tumor, improvement in the biochemical status of the patient, weight changes, etc. are of interest in cataloging the pharmacologic effects of the drug but should never be used to measure anti-tumor activity.

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Changes in the leukemias are relatively easy to measure and therefore these diseases have been a popular means of evaluating chemotherapeutic agents. However, it is important to realize that a compound which may be effective against one tumor may have no effect against others and thus it is unfortunate that so many agents have been evaluated both experimentally and clinically only against the leukemias.

It is obvious that even a large teaching hospital does not have sufficient numbers of patients with metastatic malignancy suitable for objectively evaluating many compounds except over a prolonged period of time. This circumstance has forcefully indicated the need for establishing cooperative studies in which the data of several investigative groups are combined. Five such groups have been formed or are being formed with the guidance of the Clinical Panel. Each is comparing the effectiveness of 2 agents such as Triethylenethiophosphoramide (TSPA) versus nitrogen mustard (HN) or 6-Mercaptopurine versus 6-Chloropurine according to a single protocol developed to define the patients and tumors to be studied, objective criteria of therapeutic effectiveness, and an acceptable method of randomizing the distribution of the patients in each group.

Using this cooperative study technique a statistically valid comparison of 2 compounds should be accomplished in a period of months rather than years.

To illustrate the difficulties and successes of the initial clinical trials of a chemotherapeutic drug which must be completed before a final evaluation by the cooperative study technique is rational, our investigation of Triethylenethiophosphoramide (TSPA)* and Actinomycin D** follows. Only the results of TSPA will be mentioned in detail since to date there has been no evidence of therapeutic effect of Actinomycin D against solid tumors in the adult.

Triethylenethiophosphoramide was initially given to a series of advanced cases to establish the maximum tolerated dosage and as a consequence a course of 5 daily i.v. injections of 0.2 mg/kg was proposed for a definitive study of its anti-tumor effects.

In a brief study of the effects of a second course of therapy, those patients who did not develop a hematologic depression and showed no tumor response after 4 wks were given a second course at the same dosage level or slightly higher. Immediately, 1 patient developed severe agranulocytosis and 2 others developed infections which they could not control. In none of the patients given a second course of therapy were additional favorable tumor responses noted.

Evaluation of the effectiveness of maintenance therapy was also attempted. Briefly, it was found that a weekly injection of 0.2 mg/kg intravenously was the maximum tolerated dosage and even then most patients had a continuous depression of the peripheral leukocyte count. Again, it was noted that no patient responded to this therapeutic regime who had not responded to the initial treatment.

Following these preliminary investigations a comparative study was initiated employing Actinomycin D after completing similar pharmacologic

*TSPA furnished through the courtesy of Dr. J. M. Rueggsegger of Lederle Laboratories.

**Actinomycin D furnished through the courtesy of Dr. Frederick A. Heath of Lederle Laboratories.

studies † A protocol outlining the eligibility of patients, initial chemical and hematological information to be obtained, dosages and measurement techniques was completed, and a flow sheet for follow up examinations printed. Tumor measurements were recorded weekly. The distribution of patients between the 2 agents was randomized by sealing in separate envelopes cards indicating the therapy to be employed. Random series were set up for each kind of malignancy to be studied.

RESULTS

Since the purpose of this paper is to indicate aspects of planning chemotherapeutic investigations, only a short summary of the results obtained with TSPA will be given, and no effort will be made to review the negative results in patients given Actinomycin D.

Evaluation of tumor response has been separated into 4 simple classes as indicated in Table I. Class O includes patients dying within 3 wks of treatment or given an incomplete course of therapy. Deaths have been classified as to whether the therapy was not a factor, an indirect factor, or a direct causative factor in a patient's death.

The only striking remissions seen in this study occurred in patients with breast cancer as represented by the following cases.

The wife of one of our staff members with extensive abdominal and liver metastases and rapidly accumulating ascites was unresponsive to ovariectomy and steroid therapy and was terminal before given TSPA. Three weeks later the liver rapidly reduced in size, alkaline phosphatase levels fell from 280 to 80 units, and the ascitic fluid did not recur. She was discharged from the hospital and has resumed many of her normal activities. To date this remission has lasted 3 mo. No further therapy has been given.

Three women with widespread metastases had remissions lasting about 2 mo during which time almost all of the palpable lesions completely disappeared leaving only pigmented spots to indicate the tumor site. These areas were biopsied and some were found to contain either small clumps of viable tumor cells or bizarre irradiation cells, whereas in others no tumor cells could be identified. Unfortunately, after these brief remissions the nodules reappeared rapidly and additional metastases appeared. In 2 patients a further remission of 2 mo was obtained with retreatment, but severe bone marrow depression prohibited further therapy. A fourth patient enjoyed a similar response of her soft tissue lesions, but simultaneously skeletal metastases were enlarging.

It is noteworthy that this study is not complete since too few patients have been seen to allow valid evaluation. The approximate number of patients necessary to evaluate a single compound given at a dosage level for malignancy of a single type has not been determined. Probably it will be between 10 and 20 patients with each type of tumor—a formidable number for most clinics. This again emphasizes the need for cooperative studies.

DISCUSSION

It is immediately apparent that these results differ from those reported by Bateman and co-workers.¹ However, careful study of Bateman's paper reveals that probably the majority of the excellent responses she docu-

† Preliminary data obtained from Dr. Sidney Farber.

Table 1 Triethylenethiophosphoramide Therapy

DIAGNOSIS	CLASS 0 INCALCULABLE	CLASS 1 TUMORS CONTAINING SIZE	CLASS 2 TUMORS FAILING TO INCREASE SIZE	CLASS 3 TUMORS DIMINISHING IN SIZE	CLASS 4 TUMORS DISAPPEAR COMPLETELY	DEATH NOT A FACTOR	DEATH INDIRECT FACTOR	DEATH DIRECT FACTOR	TOTAL IN SERIES
Breast	3	2		3		3		1	10
Ovary		1				1			1
Lung	3	1				3		1	4
Prostate		1							
Oral Cavity	1	6					1		1
Gastrointestinal	9					3		1	7
Melanoma								1	16
Miscellaneous		7				2		1	3
						1			7

mented were obtained by local injection rather than systemic administration. In contrast the report of Shry and Sun² is compatible with the findings of the present study. They reported objective tumor response only in patients with breast and ovarian cancer.

The toxicity of TSPA should not be minimized. Shry reported that the drug probably contributed to the deaths of 3 patients in his series of 17 patients through severe bone marrow depression. In this study, a relatively high dosage over a short period of time was chosen deliberately to obtain the maximal tumor response. Three patients died as a direct result of bone marrow depression and terminal infection and 2 others with thrombocytopenia died from hemorrhage from fungating tumor masses.

In this group of 19 patients 15 had a depression of their peripheral white cell count below 3000 and 13 of these were below 1000. In 12 patients the platelet count was depressed below 100,000 and in 6 below 50,000. Maximal depression of peripheral leukocytes occurred 2 wks following the last injection (range 5 to 27 days median 15 days and platelets 4 to 28 days median 17 days).

Actinomycin was given intravenously in daily increments to a total of 75 gamma/kg over a 5 day period. Subsequently weekly injections of 15 gamma/kg were given.

No striking tumor responses were seen nor were there any fatalities attributable to this drug. Thrombocytopenia regularly occurred soon after the therapy was completed and then returned to normal levels in a few days. Leukopenia was clinically insignificant.

CONCLUSIONS

1 Many new potential cancer chemotherapeutic chemical agents requiring clinical evaluation can be expected in the next few years. There is a great need for clinical investigators to engage in preliminary human pharmacologic studies and formal attempts to evaluate the anti-tumor activity of these compounds.

2 The only practical method of objectively evaluating chemotherapeutic agents as to anti-tumor activity in a reasonably short time involves setting up cooperative studies between clinical groups in order to pool available patients with various tumor types, utilize a common protocol so as to standardize therapy and establish rigid statistical controls.

3 The steps preliminary to acceptance of a final protocol suitable for a cooperative study are illustrated by our preliminary investigations with Triethylenethiophosphoramide.

4 Brief mention is made of the clinical results of a comparative study of TSPA and Actinomycin D. Several spectacular brief remissions were obtained in patients with metastatic breast cancer given TSPA intravenously.

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THE EFFECT OF CANCER INHIBITOR DRUGS ON THE TAKE OF WALKER CARCINOSARCOMA 256 IN RATS*

GERALD O McDONALD ERNESTO P CRUZ AND W H COLE

Metastases from primary tumors develop in numerous ways including primarily lymphatics implantation and venous emboli. The danger of implantation of cancer cells into the suture line of the colon was first emphasized by Morgan and Lloyd Davies¹. Perhaps of more importance to the patient (in intestinal cancer) is hepatic metastases which develop from venous emboli. One of us with associates² has demonstrated cancer cells in venous blood and saline perfused through the vessels supplying a carcinoma of the rectum by operation, and has emphasized the necessity of ligating the vascular trunks prior to resecting and even manipulating tumors of the colon and rectum. Fisher and Turnbull³ have carried the investigation further and report demonstration of cancer cells in the venous blood in 32 per cent of 25 carcinomas of the colon removed at surgery. We have concluded that in spite of care and caution in manipulating these tumors numerous emboli of tumor cells are discharged into the veins draining the tumor site. These cells reach the liver, some of them develop vascular attachments and form hepatic metastases. We have postulated that cells desquamated into the wound as well as venous emboli might be killed if treated by anticancer agents before they develop vascular attachments. Perhaps of more importance the microscopic metastases held in check by the patient's immunity might be killed or prevented indefinitely from growing if anticancer agents are given frequently after the tumor is excised.

This study was undertaken to determine if cancer inhibitor drugs would prevent or affect the hepatic growth of tumor cells artificially implanted into the portal circulation of susceptible animals (rats). These tumor cells would closely simulate those discharged into the portal system at the time of surgical resection of a colon carcinoma.

METHOD

The Walker rat carcinosarcoma 256 was chosen because of its hardy characteristics and its ability to grow readily when transplanted. This tumor was maintained by subcutaneous transplants in female Sprague Dawley rats weighing between 125 and 175 gm. All work was performed under aseptic operating room conditions.

The tumor cell suspension was prepared by first finely mincing the tumor and then adding a small amount of saline as recommended by Lucke and associates⁴ for producing hepatic metastases with the V₂ tumor in rabbits. This mixture was filtered through a fine stainless steel mesh producing a filtrate consisting almost entirely of single cells. A quantitative cell count was then obtained and the cell suspension diluted with physiologic saline so that 1 cc of the suspension contained between 110,000 to 125,000 cells.

Under nembutal anesthesia the peritoneal cavity of the rat was opened

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and a large mesenteric vein isolated. One cubic centimeter of the cell suspension was then injected into the isolated vein. Hemorrhage following withdrawal of the needle from the vein was controlled with gentle pressure. Groups of 10 to 20 animals were operated at one time so the tumor cell injections could be accomplished as rapidly as possible. The animals receiving injections of the inhibitor drugs received the tumor cell injections before the control groups. This was done to circumvent the possibility that if there was a decrease in the virulence of the cells after standing a longer period of time (30 to 60 min) the more virulent cells would be received by the treated group.

Three anticancer agents were used—azirserine, nitrogen mustard, and thiotepea. In the first group of animals azirserine (2.0 mg/kg of body weight) was injected into the portal circulation 1 min after the injection of the tumor cells. In the second group, nitrogen mustard (0.5 mg/kg of body weight) was injected in the same manner. In the third group, nitrogen mustard was injected into a peripheral (foot) vein 1 hr following the tumor cell injection. In the fourth group, nitrogen mustard was injected into the peritoneal cavity 1 hr following the intraportal tumor cell injection. In the fifth and sixth groups, thiotepea (2.0 mg/kg of body weight) was injected by the intraportal and intraperitoneal routes respectively, following the intraportal injection of a double strength tumor cell suspension containing 220,000 to 250,000 cells.

In each small group of 10 to 20 rats operated at the same time, half of the animals always served as controls. We felt this method eliminated as many variables as possible, the most significant variable being the different tumors used in preparing the suspension for each procedure.

RESULTS

The results obtained with azirserine were not favorable following the intraportal injection of 2.0 mg/kg of body weight 1 min after the tumor cell injection. In 42 treated and 14 control animals the incidence of tumor growth in the liver was about the same for both groups, being 95.2 per cent and 88.6 per cent respectively.

As reported previously¹ when nitrogen mustard was given in an effort to kill the injected cell, results were definitely encouraging. In a control group of 38 rats the percentage take was 97.4 per cent, whereas in 36 rats treated by injection of 0.5 mg nitrogen mustard into the portal vein 1 min after injection of cells, the percentage take was 19.4 per cent.

In another experiment in which nitrogen mustard was injected into the peritoneal cavity and still another in which the drug was injected into a systemic vein (in each case 1 hr after injection of cells) the results were almost as favorable, although the groups were smaller.

In Table 1 are listed the results of injection of 1 dose of thiotepea into the portal vein 1 min after the injection of the cancer cells. In this experiment the number of cells was increased to 225,000, doubling the number injected in the previous experiments. With the increased number of cells there was 100 per cent take in the 23 control animals. In the 27 treated animals the percentage of takes was 67 per cent.

Table 2 lists the results of 1 injection of thiotepea into the peritoneal cavity 1 hr following injection of the cancer cells in the portal vein. The

Table 1 Effect of One Dose (20 Mg/kg of Body Weight) of Thiotepe Injected Into the Portal Vein of Rats One Minute After Injection of the Cancer Cells

GROUP	NO. RAT	PER CENT TAKES
Treated	27	67.0%
Control	23	100.0%

Table 2 Effect of One Dose (20 Mg/kg of Body Weight) of Thiotepe Injected Intraperitoneally One Hour After Injection of Cancer Cells Into the Portal Vein

GROUP	NO. RAT	PER CENT TAKES
Treated	30	14.3%
Control	30	75.9%

percentage of takes was 75.9 per cent in 30 control animals and 14.3 per cent in 30 treated animals

DISCUSSION

Prior to obtaining the results recorded herein it was necessary for us to demonstrate the feasibility of injecting 1 cc of solution into the relatively small mesenteric vein of the rat without complications of spillage and hemorrhage. Following this we had to determine if the Wilker 256 tumor would grow in the liver following portal vein injection of a tumor cell suspension. Lucke and Breedis¹ had shown the liver to be a favorable site of growth of the rabbit V₂ carcinoma when a cell suspension of this tumor was injected into the portal vein. Using their method in preparing the cell suspension, modifying it to obtain a suspension of single cells, we were able to show a high percentage of takes in the liver following the intraportal injection of a suspension containing 100 000 to 150 000 cells. There were some deaths from anesthesia and hemorrhage. These animals were discarded from our series.

Admittedly these series are not large, yet the results appear to be consistent in all groups. The control rats tended to die by the 21st postoperative day and their livers contained multiple tumor nodules. In comparison those treated rats which did develop tumor had fewer and much smaller tumors than did the control animals. However from our results we wished primarily to learn the effectiveness of the inhibitors in completely preventing, not just decreasing hepatic growth.

We realize the difficulty in transferring the results of animal experimental work to human clinical procedures. However the results of this experimental animal work have been sufficiently encouraging to stimulate us to give nitrogen mustard to patients having resection of tumors which metastasize by vein as well as by lymphatics. We have been giving a 4 day course of nitrogen mustard, the first dose of 0.1 mg/kg of body weight being given the day of operation. In intestinal tumors we have given the first dose into the peritoneal cavity. To date our maximum dose to freshly

operated patients has been 60 mg at present we are limiting the therapy to patients under 71 yr of age

SUMMARY

Results of prophylactic anticancer therapy are reported herein. The experimental method of injecting a tumor cell suspension into the portal vein of rats was chosen because it resembled the mechanism of production of hepatic metastases in carcinoma of the digestive tract. When nitrogen mustard was injected into the portal vein into the peritoneal cavity or into a peripheral vein it was effective in decreasing the incidence of take of the Walker carcinosarcoma cells following their portal vein injection in the rat. Thiotepea was also effective in decreasing the incidence of tumor takes in the liver when injected intraperitoneally whereas it was only moderately effective when injected directly into the portal vein. All series are small and are being enlarged. Azaserine when similarly injected into the portal vein was not effective in preventing tumor growth in the liver.

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HORMONAL FACTORS AFFECTING GROWTH OF EXPERIMENTAL SECONDARY BONE TUMORS*

T. B. SINGH, STANLEY C. SKORYNA, A. C. RITCHIE, AND D. R. WEBSTER

The investigations during the past 10 yr have shown to an increasing extent the significance of various systemic factors which affect the process of malignancy. The promoting and inhibitory action of hormonal factors is evident in many clinical and experimental tumors. In this experiment it was attempted to evaluate the effects of estrogens, androgens and cortisone preparations commonly used in the treatment of metastatic bone disease.

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on the intraosseous transplants of Walker Carcinoma 256 a highly anaplastic mammary carcinoma of rats

METHOD

Hooded male rats of the Royal Victoria Hospital strain weighing 120 to 150 gm were used. Male animals were selected because of metabolic variations in the females caused by sexual cycle. The Walker Carcinoma 256 was transplanted from Sprague Dawley strain to our colony using a standard intramuscular transplantation for comparison with the growth of the tumor in intraosseous implants.

Intraosseous implantation was performed using the following method. The animal was anesthetized with nembutal and a transverse skin incision of 1 cm length was made on the anterior surface of the left knee joint. The extensor tendon and the anterior capsule of the knee joint were transected and the lower end of the femur exposed. Using a dental burr No. 7 a hole was drilled in the lower end of femur parallel to the shaft and about 3 to 5 mm deep. A tumor implant weighing 10 mg was placed in the opening. Subsequently the burr hole was occluded with a stainless steel screw. The extensor tendon and skin were resutured. Animals tolerated the procedure well and no operative mortality was encountered.

Intraosseous implantation was performed in 144 rats and the animals were divided into 4 equal groups. Group 1 (Control). No hormones administered. Group 2 (Cortisone). Intramuscular administration of Cortogen' (Schering) 5 mg/day (0.05 mg/gm of body weight) from the day of implantation. Group 3 (Estrogens). Intramuscular administration of Premarin (Ayerst) 2 mg/day (0.016 mg/gm of body weight) from the day of implantation. Group 4 (Androgens). Intramuscular administration of Oreton (Schering) 5 mg/day (0.05 mg/gm of body weight) from the day of implantation.

Twelve rats from each group were sacrificed each week. Postmortem examination and a search for metastases in other organs was carried out in all animals. The femur containing the tumor implant was fixed in 10 per cent formalin solution, decalcified in Sequestren Na_4 and sectioned longitudinally at 5μ in thickness.

RESULTS

A take was considered positive if any evidence of malignant tissues was found in the section. The invasiveness of the tumor implant was judged by the destruction of the adjacent bony tissues. The resistance of various layers of bone to tumor implant was studied. The epiphyseal cartilaginous layer of the lower end of the femur appeared to be a significant barrier to the growth of the tumor but once the tumor implant crossed the cartilage zone it spread relatively easily into the marrow cavity and destroyed the trabeculae of spongy bone and marrow cells. The compact bony layer was more resistant to tumor invasion than the spongy layer and marrow cavity.

1. The Control Group showed 77.7 per cent takes of the implants of the Walker tumor with moderate extension of the tumor and moderate reactive bone formation.

2. The Cortisone Group showed a 94.4 per cent take of the implants



Fig. 1 (Control) Walker carcinoma 256 growing in the marrow cavity $\times 100$ H & E



Fig. 2 (Cortisone) Necrosis in Walker carcinoma growing in the marrow cavity $\times 100$ H & E



Fig. 3 (Estrogen) Walker carcinoma invading marrow tissue $\times 400$ H & E



Fig. 4 (Androgen) Fibrosis of the marrow cavity around invading Walker carcinoma $\times 400$ H & E

At 7 days the implant showed more extension than the controls but at 14 days the degenerative changes and necrosis were observed and at 21 days necrosis was more frequently observed and more extensive than in any other group. The formation of reactive bone was not affected by the cortisone in the dosage used in this experiment.

3 The *Estrogen Group* had 88.9 per cent takes of the implants. Enhancement of the invasiveness of the tumor implants was observed in 25 out of 36 rats. Reactive bone formation was slower than the control group.

4 The *Androgens Group* showed the lowest 'take' (55.5 per cent). The extension of the tumor was considerably less than in the controls. A zone of fibroblastic and osteoblastic proliferation was observed around the implants in the majority of animals in this group. This group showed more reactive bone formation than any others.

DISCUSSION

Effects of Hormones on Secondary Tumor Growth in Bone Tissue. The bone tissue and the transplanted tumor tissue may be affected in a different manner by the same hormonal stimulant. This fact probably explains the effects of hormones used in this experiment on the growth of intraosseous transplants of Walker Carcinoma 256. The final result depends on the various inhibitory and promoting effects, the local resistance to tumor growth and the malignant potentialities of transplanted tumor tissue.¹

The estrogen treated animals showed less bone formation than the controls. This might be partially responsible for the enhancement of tumor growth observed in this group. That mammary carcinoma can be induced experimentally in rats by administration of estrogens and that tumor growth can be enhanced by estrogen administration² suggests that estrogens may be the most important dependency factor in mammary cancer. However, as estrogens have different effects on bone in various species the data should not be extrapolated to other species.³

The effect of cortisone on bone is probably non specific and secondary to other systemic effects. Even in high doses it does not affect bone metabolism except indirectly by loss of protein nitrogen from the body. In this experiment the highest take of intraosseous implants was observed in the cortisone treated group. The tumor had also more tendency to spread during the first and second week. It is possible that cortisone promoted the tumor growth during the period perhaps by suppressing the antigenic reaction. The necrosis and regression of tumor transplants seen at later periods might be due to the suppressive effect of cortisone on pituitary hormone production which in turn reduces estrogen production. This effect of cortisone on intraosseous implants differs from that on subcutaneous transplants which exhibited decreased growth rate from the beginning.⁴

Androgens have a promoting effect on osteogenesis. It is likely that increased bone growth increased the resistance to the invasion of the implanted Walker Carcinoma and might have been responsible for regressive changes and decreased growth rate observed in the tumor implants in this experiment. On this basis androgens might be expected to produce objective improvements in patients with bone secondaries from breast carcinoma. However, the possibility that androgens neutralize the effects of endogenous estrogens should also be considered. It may be relevant that Bethune observed that in patients with breast cancer the prognosis after hypophysectomy was better in cases with bony metastases than in those with involvement of the soft parts.

Tumor Growth in Bone Tissue. The study of the effects of hormones on intraosseous implants of Walker Carcinoma 256 permits also certain conclusions as to the tumor growth in general. This experiment demonstrates that the secondary tumor growth is more restricted in osseous tissue than in muscle tissue. On intramuscular transplantation the tumor grows to a palpable size after 1 wk. and by the third week has enlarged sufficiently to produce cachexia and death of the host. The takes are almost 100 per cent. On the intraosseous implantation the tumor does not extend more than 1 to 1.5 cm. inside the marrow cavity during a corresponding period of time. This inhibition of the expansion of the tumor appears to be related to

resistance of bony tissue to invasion due to some local inhibitory factors

The resistance of various components of bony tissue to tumor invasion varies. As observed in this experiment the growing epiphyseal cartilage appears to be most resistant to invasion by malignant cells and the compact bone to be considerably more resistant than spongy bone. The resulting growth pattern of the tumor therefore differs from that in soft tissues.

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COMBINED THERAPY (BILATERAL ADRENALECTOMY AND RADIOACTIVE PHOSPHORUS) FOR CARCINOMA OF THE BREAST WITH WIDESPREAD METASTASES TO BONE*

HARVEY KRIEGER WILLIAM E. ABBOTT WILLIAM D. HOLDEN
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In 1952 Huggins¹ demonstrated that bilateral oophorectomy and adrenalectomy were effective forms of palliative treatment for patients with widespread metastases from carcinoma of the breast. It has now become apparent from the various reports in the literature that only 40 per cent of the patients have tumors which are estrogen dependent and therefore derive significant benefit from the aforementioned procedures.^{2,3} Although various criteria and methods for the selection of patients have been recommended, it is impossible in any individual case to predict the magnitude and duration of palliation. When there are extensive and widespread metastases to bone, it is not possible to treat all the various sites by external irradiation. However, the availability of radioactive isotopes offers an opportunity for internal irradiation. Friedell and Storaasli⁴ in 1950 reported a series of 12 patients with widespread metastases to bone who were treated with radioactive phosphorus (P^{32}). This isotope has a physical half life of 14.3 days and emits a beta particle which has an average range of 2 mm. Therefore the radiation is confined primarily to the tissue in which it is deposited. Since bone con-

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DISCUSSION

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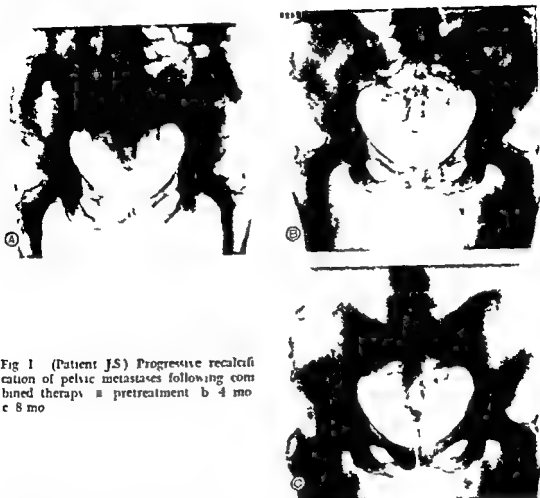


Fig 1 (Patient JS) Progressive recalcification of pelvic metastases following combined therapy: a pretreatment b 4 mo c 8 mo

the combined therapy and has been able to carry on her occupation as a school teacher as well as participate in such activities as gardening, hiking and swimming. Two of the patients (JS and LPr) had no evidence of visceral metastases at the time of operation. They are asymptomatic 9 and 29 mo respectively after therapy. The other 3 patients (VG, MMc, and IPe) all had visceral metastases at the time of operation. VG and MMc died 18 and 11 mo after therapy, although they both had objective improvement in their osseous metastases and led normal active lives until shortly before death. LPe is living 10 mo after therapy but now has ascites and her liver function tests show abnormalities which are compatible with hepatic metastases. It is apparent that the prognosis is much better in those patients who at the time of therapy have their metastases confined to the skeletal system.

The total amount of radioactive phosphorus given to each of the 5 patients was comparable to that employed by Friedell and Storarsli.⁴ Purpura occurred as a complication in 9 of their 12 patients. The platelet count fell to 50,000 or less in 6 of their patients and a depression of leukocytes to 3,000 or less occurred in 5 patients. Purpura has not occurred in any of the 5 patients treated with P^{32} after adrenalectomy, nor was there any significant prolonged depression of the platelet or leukocyte counts. Table 1 lists the hemogram for each of the 5 patients before they received P^{32} as well as the maximum change in the hemogram following the full course of therapy. The

tains more phosphorus than any other tissue, the major fraction of any administered P^{32} will accumulate in the skeletal system. This has been demonstrated both experimentally and in tissues obtained at autopsy from a patient treated with P^{32} .¹ Seven of the 12 patients who were treated with P^{32} had significant relief from bone pain and weakness. Two patients had significant objective improvement in their osseous lesions demonstrable by recalcification as shown by serial roentgenograms. The only complication of importance which occurred with this form of therapy was severe depression of the hematopoietic system.

When several patients who had previously been adrenalectomized were given comparable amounts of P^{32} , it was noted that there was no significant depression of the hematopoietic system. Therefore it was decided to determine whether there was any significant difference in the uptake, distribution and excretion of P^{32} before and after adrenalectomy.

METHOD

Six patients with widespread osseous metastases who were to undergo adrenalectomy and oophorectomy were admitted to the Metabolic Division of the University Hospitals of Cleveland for this study. Three days before adrenalectomy a tracer dose of radioactive phosphorus (0.5 to 1.0 mc) was administered intravenously. All urine and feces were collected in 24 hr periods up to the morning of operation. At the time of adrenalectomy a segment of rib containing both metastatic tumor tissue and normal bone was obtained for assay. Two to 4 wks after adrenalectomy a second tracer of P^{32} was administered and again urine and feces were collected for 3 days and on the fourth day a segment of rib similar to that removed at the time of adrenalectomy was obtained.

Isotope assay methods. *Urine.* Three 1 ml aliquots of each 24 hr urine collection were pipetted into metal planchets, dried and counted with a Geiger Muller counter. *Feces.* Each 24 hr collection of feces was brought to a known volume with distilled water, wet ashed with concentrated nitric acid, and 1 ml aliquots placed in planchets, dried and counted. *Bone.* Bone samples were wet ashed with nitric acid, weighed and counted in the same manner as the urine and feces.

After the second tracer study was completed the patients were given a therapeutic course of P^{32} . This consisted of 2 mc weekly until a total dosage of 9 to 18 mc was given. Complete blood studies including hemoglobin, red blood cell, white blood cell and platelet counts were obtained prior to the treatment with P^{32} and periodically thereafter.

RESULTS AND DISCUSSION

Five of the 6 patients included in this study were given a therapeutic course of P^{32} after adrenalectomy. These 5 patients improved subjectively as manifested by diminution or complete disappearance of bone pain and increase in strength and appetite. Four patients who had been bedridden before adrenalectomy were subsequently able to carry on all of their normal activities. Objective improvement in all 5 of these patients was evident by progressive recalcification of osseous lesions as shown by serial roentgenograms. Figure 1 shows the progressive recalcification of the pelvic metastases in patient J.S. over an 8 mo period. This patient had striking benefit from

Table 2 Tissue Uptake of P^{32} Before and After Adrenalectomy

PT	TYPE OF TISSUE	BEFORE	AFTER
		ADRENALECTOMY	ADRENALECTOMY
		COUNTS/MIN/CM	
M M	Normal Bone	3461	5315
	Tumor Adjacent Bone	9715	10516
	Tumor Tissue	22606	25153
J S	Normal Bone	26736	19519
	Tumor Adjacent Bone	50753	36861
	Tumor Tissue	39589	96237

tissue were assayed normal bone bone adjacent to metastatic tumor and tumor tissue. It should be noted that in both patients pre and postadrenalectomy the uptakes of P^{32} in tumor tissue and bone adjacent to tumor was significantly greater than the uptake by normal bone. This agrees with the findings of Friedell and Storavik.⁴ Following adrenalectomy in patient J S the ratio of P^{32} uptake in tumor tissue as compared to the uptake in normal bone was significantly increased above the ratio of uptake in these tissues before operation.

SUMMARY

1 Five patients with widespread metastases to bone from carcinoma of breast were given a therapeutic course of P^{32} following bilateral oophorectomy and adrenalectomy.

2 All 5 patients were significantly improved both subjectively and objectively with respect to their bone metastases and none had any clinically significant depression of the hematopoietic system.

3 Although to date we have been unable to demonstrate conclusively a significant difference in the distribution of P^{32} before and after adrenalectomy we believe that the administration of a course of P^{32} after adrenalectomy provides a maximal form of palliation for the patient with widespread metastases to bone from carcinoma of breast.

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Table 1 Hemograms Before and After Treatment for 5 Patients Who Received Therapeutic Course of P³² After Adrenalectomy

PATIENT	AGE	P ³² DOSE		Hgb	HEMOGRAM		
				GRAMS	RBC x 10 ⁶	WBC	PLATELETS
V G	40	16 mc in 67 days	I **	14.1	4.63	4,400	
			M*	10.6	3.53	2,150	145,080
I I r	69	15 mc in 57 days	I	13.7	5.33	5,300	400,000
			M	12.8	1.30	2,950	135,000
M Mc	52	9.2 mc in 31 days	I	9.0	3.17	4,850	128,990
			M	8.9	3.17	3,050	66,880
I I e	36	16 mc in 53 days	I	11.9	1.00	9,500	545,160
			M	11.1	3.86	5,850	265,230
J S	50	18 mc in 36 days	I	14.5	4.70	5,600	400,900
			M	12.5	4.14	4,300	66,240
Average	49	14.8 mc in 44.8 days	I		4.36	5,930	368,762
			M		3.80	3,660	195,486
Average (Friedell & Storaasli)	48	16.0 mc in 41.5 days	I		3.88	8,800	307,000
			M		3.15	2,000	90,000

**1 retreatment Hemogram

* Maximal change after P³² treatment

Average values for the 12 patients treated by Friedell and Storaasli are also shown in Table 1. The difference in the magnitude of the depression of the hematopoietic system in these 2 groups of patients suggested the possibility of some change in the uptake, excretion or distribution of P³² following adrenalectomy.

Two of the patients (J S and L Pe) showed hypercalcemia and negative calcium balance (600 to 800 mg daily urinary calcium with an intake of 300 mg). The calcium balance of both of these patients became positive and their serum calcium concentrations were restored to normal following adrenalectomy.

It was thought that if the rapid and progressive bone destruction were reduced or eliminated following adrenalectomy increased amounts of radioactive phosphorus might be deposited in metastases in association with the recalcification of these lesions. In order to investigate these possibilities P³² excretion and tissue uptake studies were undertaken.

The excretion of P³² in the urine and feces over a 3 day period was essentially the same preoperatively and 2 to 4 wks postoperatively indicating no significant change in the retention of P³² after adrenalectomy in this group of 5 patients.

An attempt was made to determine whether there was a more selective uptake of P³² by tumor tissue as compared to normal bone after adrenalectomy. Satisfactory rib segments were obtained for assay from only 2 of the patients before and after adrenalectomy. Table 2 shows the uptake in these rib segments expressed as counts/min/gm of wet tissue. Three types of

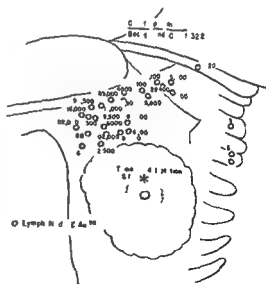
apparatus of their internal mammary lymph nodes, 1 had carcinoma involving axillary and one or more internal mammary lymph nodes and 3 had involvement of axillary lymph nodes only. No patient had carcinomatous deposits in the internal mammary lymph nodes alone.

DISCUSSION

Distribution of Radiogold in the Absence of Demonstrable Metastatic Neoplasm Regardless of the site of placement of the radiogold there was usually a generalized distribution in identifiable lymph nodes. Of the 332 lymph nodes examined in these 20 patients, only 60 were not found to contain any radiogold.*

Although in some individuals the lymph nodes nearest the injection site contained the greatest concentration of radioactive particles there were many instances in which the highest node in the axilla or one of the nodes along the lateral portion of the axillary vein or in the region of the subscapular vessels had an equally high count. Considerable variation in counts per node was quite common and this could occasionally but not always be correlated with the size of the lymph node. Lymphatic flow to the internal mammary lymph nodes was merely a trickle as compared with that to the axillary chain. Although a comparison of the gold deposited in axillary and internal mammary lymph nodes is most gross the internal mammary lymph nodes contained less than 1 per cent to 2 per cent of the total radiogold deposited.* Even if it is assumed that the maximum number of internal mammary nodes were present and that the lymph nodes examined were representative these figures would not be appreciably altered. It was quite clear that injection in any quadrant could be followed by the deposition of gold in the internal mammary chain. This distribution pattern as well as estimates of quantitative flow are similar to the observations of Hultborn.²

Fig 1 Location and activity of 27 lymph nodes removed from a radical mastectomy specimen in a patient receiving 164 mc of radiogold 2½ hrs before surgery. Note the variation in activity of the various nodes. This could not be specifically correlated with size of the lymph node. In this instance the counts in the internal mammary nodes removed represent approximately 02% of total lymph node activity. None of the lymph nodes contained carcinoma.



The criterion for deposition of radiogold in a lymph node was a count of at least twice background.

* There was one exception to this observation in that a patient with a medially situated lesion had 19 lymph nodes examined, 2 of which were internal mammary nodes and contained 1½ per cent of the radiogold deposited.

PROGRESSION OF LYMPHATIC METASTASES IN CARCINOMA OF THE BREAST*

COLIN G THOMAS JR

The surgical treatment of carcinoma of the breast has been based upon the premise that this neoplasm spreads primarily by the lymphatic route—a centrifugal embolic spread with progressive chain like involvement of more lymph nodes. This concept plus the clinical evidence that there may be a segmental type of lymphatic flow from certain areas of the breast, has led to the extension of surgery beyond axillary node dissection in an effort to include a greater area of potential lymph drainage. The findings of carcinomatous lymph nodes in these areas of extension has been considered justification for this approach. The validity of these concepts however has never been carefully scrutinized experimentally since until recently most of the views on lymphatic drainage of the breast were based upon studies in the cadaver and the location of metastatic cancer. These methods have not permitted a quantitative study of lymphatic flow and it has not been possible to appraise the relative importance of the various routes or under what conditions the accessory pathways may assume greater import. Furthermore relatively little attention has been given to the alteration in lymph dynamics that occurs in the presence of metastatic neoplasm. The purpose of this investigation was to make a quantitative study of lymphatic flow from various quadrants of the breast and in particular to appraise any significant alteration in lymph dynamics by the presence of lymph node metastases. Radioactive colloidal gold which has been demonstrated to follow lymphatic pathways and to become phagocytized by the reticuloendothelial cells was selected for this study.¹

METHOD

Tracer amounts of radiogold (0.1 to 1.0 mc) in a volume of 1 ml were injected into large tumors or adjacent smaller tumors in patients undergoing radical mastectomy. In most instances injection was performed the day preceding surgery although in a few cases the time interval was as short as 1 hr. Evaluation of the first group of patients did not include examination of the internal mammary lymph nodes. More recently however one or more internal mammary lymph nodes has been removed from the second, third or fourth intercostal space as a diagnostic aid. Examination of the surgical specimen was carried out within 24 hr. All grossly identifiable lymph nodes being removed by sharp dissection. In addition to determining their radioactivity the location of all nodes was charted and histologic examination was made for relative amounts of lymphoid elements and neoplasm.

Thirty three patients with breast cancer were studied by these methods. Twenty patients had no evidence of lymph node metastases and these included 8 with internal mammary node dissections. Thirteen patients including 7 with internal mammary node dissections were found to have 1 or more metastatic lymph nodes containing cancer. Of the 13 patients having an

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tion in lymphatic flow such as to the opposite breast diaphragm or hepatic lymphatics. In these individuals there may be no demonstrable gold in axillary or internal mammary lymph nodes even though they contain lymphatic tissue as well as carcinomatous.

From these observations in patients with carcinomatous nodes it is evident that considerable alteration in normal routes of lymphatic flow is common as reflected by a) abnormal distribution of radiogold deposition b) the lack of radiogold in lymph nodes containing lymphatic tissue as well as cancer and c) a demonstration of radiogold in sites other than axillary and internal mammary lymphatics. The most significant of these alternate pathways remains to be determined.

CONCLUSIONS

If the dissemination of radiogold can be used as a guide to lymphatic flow from the breast it is evident that particulate matter in any segment of the breast may pass through any of the lymphatics known to drain breast parenchyma. Despite the clinical evidence that suggests some segmental lymphatic drainage from the breast⁴—the predominant lymphatic flow from all areas as herein studied is toward axillary lymph nodes. Less than 1 to 2 per cent of lymphatic flow is in the direction of the internal mammary lymphatics. It is likely that other accessory pathways have similar import.

Conditions are quite different and somewhat unpredictable once obstruction of some of the afferent lymphatic pathways occurs. Where axillary lymph nodes are extensively involved by cancer internal mammary infra-mammary and all accessory routes of lymphatic flow are utilized. In the presence of axillary lymph node metastases it is easy to understand the high incidence of internal mammary metastases. It should be emphasized that whereas the axilla probably remains a more effective barrier against lymphatic spread of carcinoma because of what constitutes more or less multiple lines of defense the internal mammary lymph nodes are few, small and probably provide a most ineffective barrier against carcinomatous spread.

These studies would seem to have the following clinical significance. In the absence of lymphatic metastases to axillary lymph nodes the inclusion of the internal mammary lymphatic chain in an effort to encompass the regional lymph flow from the breast enhances this possibility only by the percentage of lymphatic flow to this area. This probably represents less than 2 per cent of total lymphatic flow. It is likely but has not been substantiated experimentally that the quantitative flow to this area from medial portions of the breast may be somewhat higher.

In the presence of axillary lymph node metastases particularly when they occur to such an extent that there is an obstruction in afferent lymphatics and alteration in lymphatic flow the internal mammary lymphatic chain represents only one of the alternate routes available—many of which are unpredictable. Extirpation of the internal mammary chain under these circumstances would seem to provide a relatively insignificant removal of potential areas of lymphatic drainage.

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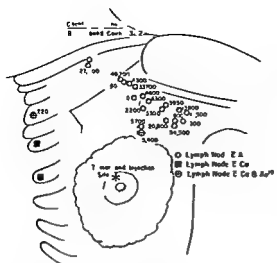


Fig 2 Location and activity of 23 lymph nodes removed from the surgical specimen of a patient with a neoplasm located immediately superior and slightly medial to the nipple. All 3 internal mammary lymph nodes contained carcinoma with two completely replaced by neoplasm and the third nearly so. The involved axillary node still retained considerable phagocytic function despite partial replacement by cancer.

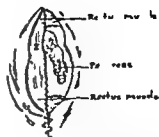
A contributing factor to the relatively minor roles played by the internal mammary lymphatics may be the small number as well as the small size of the lymph nodes present. This would in effect produce a more ineffectual barrier and one that is soon replaced by neoplasm.

From these studies in patients without metastases and employing radiogold as a criterion of lymphatic flow, it is evident that there is very little segmental lymphatic flow from quadrants of the breast. All sectors drain primarily by way of the axillary lymph nodes, but with some drainage to internal mammary nodes. There was a suggestion that the internal mammary nodes are perhaps relatively more important in receiving afferent lymphatics from the medial portions of the breast. However, even here lymphatic flow is primarily in the direction of the axilla.

Lymphatic Drainage as Altered by the Presence of Carcinomatous Lymph Nodes. In evaluating the location of lymph nodes as well as the radiogold in patients with one or more lymphatic metastases, several findings were of interest. The presence of carcinoma in the internal mammary lymphatics was always associated with axillary metastases. The location of the axillary metastases did not reflect the location of the primary tumor, although in general the nodes of Rotter were most frequently involved with lateral quadrant lesions. In several instances, what would be considered the nodes nearest the tumor were uninvolved, whereas only the apical axillary nodes contained carcinoma. This skipping of lymph nodes has also been noted by Monroe.³ The presence of carcinoma was associated with a haphazard distribution of radiogold through the uninvolved lymph nodes, as compared with the distribution in patients without metastases. This irregular distribution of radiogold in uninvolved lymph nodes can best be explained on the basis of lymphatic blockage. There are not a sufficient number of observations to be able to indicate a quantitative relationship between the number of lymph node metastases and alteration in lymphatic flow. It is somewhat difficult to reconcile this observation with the fact that in many individuals all axillary lymph nodes are involved with carcinoma unless this involvement represents a retrograde permeation rather than embolic extension.

The presence of more extensive axillary lymph node involvement by neoplasm but without any demonstrable lymphedema results in marked altera-

Fig. 1



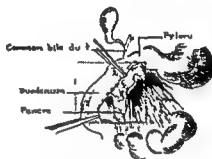
STAGE I

Division of the left limb of the pancreas

Elevation of the left limb to the rectus compartment with blood supply intact



Division of the blood supply to the transplant from below the peritoneum



STAGE II

Incision of the remaining pancreas and adjacent segment of the duodenum

islet cells and then observing whether the tissue would survive auto transplantation

METHOD

Stage I (see Fig. 1) A left paramedian incision was made under intravenous nembutal anesthesia. The rectus sheath was opened widely and approximately 7 cm. of the rectus muscle excised. The upper and lower borders of the rectus compartment were then closed thus creating a fascial envelope into which the transplant could be placed. The peritoneal cavity was then opened and the left limb of the pancreas mobilized preserving several of the vessels entering the gland from the splenic pedicle. The gland was then transected as closely as possible to its most central part. The major pancreatic duct could be seen easily in most cases and was suture ligated. The isolated left limb of the pancreas was then mobilized out of the peritoneal cavity taking great care not to tear off the vessels nourishing it from the splenic pedicle. The peritoneum was closed about the vessels supplying the transplant exercising care not to damage or constrict these vessels. This step in the procedure was extremely critical for too much tension would produce ischemia of the gland. The previously prepared rectus compartment was then closed about the transplant. Postoperatively the dogs were given tetracycline banthine and nothing but water by mouth for 48 hr. Wound edema was usually present for 3 or 4 days but then rapidly subsided. Banthine was used because of its suppressive effect on pancreatic secretion. After 7 days the dogs were fed standard kennel diets and awaited Stage II.

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ISLET CELL TRANSPLANTATION*

WILLIAM R. RUNDLES AND HENRY SWAN

Transplantation of the pancreas or of functioning islet cell tissue has long been envisioned as a way of surgically curing diabetes mellitus. To date the problems relative to homografting any tissue have been insurmountable save in special instances such as gamma globulinemia,¹ monozygotic twins, closely inbred animals, arterial and corneal transplants, and in certain endocrinologically active tissues such as parathyroid.

Autografts of various tissues, i.e., thyroid and adrenals^{2, 3} have been shown to be successful when implanted in various sites such as muscle, subcutaneous space, liver, spleen, brain, and serosa of gastrointestinal tract.

The object of this investigation was to determine whether functioning islet cells, devoid of pancreatic acinar tissue, could be transplanted in the same animal. A thorough survey of available literature has failed to reveal any reports of transplantation of islet cells *per se*.

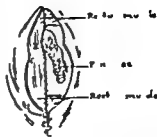
The classic work of Von Mering and Minkowski⁴ demonstrated that a transplant of part of the pancreas outside the peritoneal cavity, or separated from the duodenum but with its blood supply intact, could prevent the development of diabetes after removal of the abdominal pancreas. They then removed the transplant and found that glycosuria occurred. Later attempts to transplant portions of the pancreas were unsuccessful because of the profound autolytic effect of the pancreatic digestive enzymes. Efforts to prepare an active extract from pancreatic tissue with which to treat diabetic patients were also unsuccessful until Bunting and Best ligated the pancreatic duct to produce atrophy of the acinar tissue and subsequently prepared an active extract.

This method of ligating the pancreatic duct to produce acinar atrophy has been adapted for obtaining pancreatic tissue for transplantation. The significant feature of this approach is that the pancreatic autograft is essentially devoid of acinar cells yet contains active islets.

The following experiments on 18 dogs were performed in the form of staged operations with the objective of first producing a preparation of

*From the Department of Surgery and The Harkness Experimental Laboratory, University of Colorado School of Medicine, Denver, Colo. Aided by a grant from The National Institutes of Health, United States Public Health Service (GG 3029).

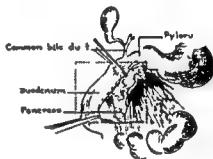
Fig. 1



STAGE I

Division of the left limb of the pancreas

Elevation of the left limb to the rectus compartment with blood supply intact



STAGE II

Division of the blood supply to the transplant from below the peritoneum

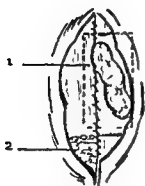
Excision of the tumor, pancreas and adjacent segment of the duodenum

islet cells and then observing whether the tissue would survive autotransplantation

METHOD

Stage I (see Fig. 1) A left paramedian incision was made under intravenous nembutal anesthesia. The rectus sheath was opened widely and approximately 7 cm. of the rectus muscle excised. The upper and lower borders of the rectus compartment were then closed thus creating a fascial envelope into which the transplant could be placed. The peritoneal cavity was then opened and the left limb of the pancreas mobilized preserving several of the vessels entering the gland from the splenic pedicle. The gland was then transected as closely as possible to its most central part. The major pancreatic duct could be seen easily in most cases and was suture ligated. The isolated left limb of the pancreas was then mobilized out of the peritoneal cavity taking great care not to tear off the vessels nourishing it from the splenic pedicle. The peritoneum was closed about the vessels supplying the transplant exercising care not to damage or constrict these vessels. This step in the procedure was extremely critical for too much tension would produce ischemia of the gland. The previously prepared rectus compartment was then closed about the transplant. Postoperatively the dogs were given tetracycline banthine and nothing but water by mouth for 18 hr. Wound edema was usually present for 3 or 4 days but then rapidly subsided. Banthine was used because of its suppressive effect on pancreatic secretion. After 7 days the dogs were fed standard kennel diets and awaited Stage II.

Fig. 2



STAGE III

1 Excision of the transplant
2 Transplant is transferred to the right lower quadrant in thin slices



STAGE IV

Excision of the transplanted islet cells to again produce diabetes

Stage II (see Fig. 1) In approximately 6 to 8 wks Stage II was performed hoping that by this time most of the scar tissue of the transplant would be atrophic. A right paramedian incision was utilized, the abdomen opened and the vascular pedicle going to the left upper quadrant transplant was sectioned. A thorough search of the abdominal cavity was made at this time for aberrant pancreatic tissue. A complete pancreatectomy was then performed removing all intraabdominal pancreas. This necessitated in all cases excision of a segment of duodenum because of the interrelationship of the blood supply of the right limb of the pancreas and the duodenum. A conventional type duododuodenostomy was performed just distal to the entrance of the common bile duct.

Postoperatively these dogs were given prophylactic penicillin and streptomycin for 1 days and not allowed food until stools were passed. Urine was tested with clinitest daily, and a non fasting blood sugar was done during the first post op week to insure absence of hyperglycemia and glycosuria. Stertorrhea developed almost immediately and was combatted with Viokase * Entozyme ** tween 80 and a high protein low fat diet with supplemental vitamins and amino acids.

Stage III (see Fig. 2) Within the first 2 wks following Stage II, on dogs that did not become diabetic the next procedure was carried out. This consisted of an en bloc excision of the entire left upper quadrant to insure complete removal of the transplant. This necessitated complete removal of the entire abdominal wall down to and including peritoneum. The transplant was then sectioned into thin (less than 3 mm) slices and implanted into the lower portion of the right rectus compartment with marking sutures placed for later identification. This tissue when examined microscopically showed numerous islet cells and varying degrees of degeneration of the acinar tissue. It was quite difficult to secure complete degeneration of all of the acinar elements. The defect in the left upper quadrant could be closed in most cases by pulling together the tissue at hand.

Immediately following the Stage III operation all dogs immediately became diabetic and the postoperative care then revolved about the treatment

*Viokase (whole raw pancreas) Violin Corp. Monticello Illinois

**Entozyme (pepsin pancreatin bile salts) A H Robins Inc. Richmond Va.

of the diabetes and the continued nutritional problems caused by the steatorrhea. All dogs received regular insulin administered on the basis of urine sugar and blood sugar determinations for the first 24 to 48 hr. After this they were switched to NPH insulin given twice daily prior to their twice-daily feedings. Close regulation of the diabetes was attempted. At intervals insulin dosage was cut sharply to provide a stimulus for autogenic insulin production.

Steatorrhea was very troublesome in all dogs which had been pancreatectomized. After trying some of the usual pancreatin preparations and fat emulsifiers without satisfactory results, a combination was finally found which seemed to be fairly effective in decreasing steatorrhea. This consisted of adding 1 to 2 gm. of Viokase and 2 Entozyme tablets to each feeding. A high protein, low fat diet was given and the Viokase powder mixed well with the dog food. Somagen*** granules were also added to each feeding plus multiple vitamins and iron. Parenteral vitamin B complex and vitamins A and K were also given at intervals.

With this routine stools could be reduced to 2 or 3 copious semi-fatty ones per day. Despite this reduction in steatorrhea a gradual weight loss occurred.

Stage IV (see Fig. 2). The final step in the experiment was the excision of the iso-transplant in the right lower quadrant to again produce total diabetes.

RESULTS

Eighteen dogs underwent elevation of the left limb of the pancreas into the rectus compartment. Three dogs died in the early postoperative period from wound eversion or peritonitis.

Fifteen dogs underwent complete pancreatectomy (Stage II). Of these dogs (Group I) 7 were *not* diabetic indicating that the transplant was producing insulin. Eight dogs (Group II) were definitely diabetic after pancreatectomy indicating that the transplant had failed to survive or function. The failures were presumed due to interference with the blood supply to the transplant in the first stage procedure. These 8 diabetic dogs in Group II served as controls and all demonstrated a well known sequence of events related to acute total pancreatectomy consisting of anorexia, vomiting, extreme weakness, cachexia and lassitude leading to death within 10 days if not given insulin. This typical picture in the acutely depancreatized dog is quite characteristic.

Of the 7 dogs (Group I) that did *not* become diabetic following pancreatectomy, 2 dogs died before the next stage could be done, 1 from peritonitis and 1 from distemper.

The remaining 5 dogs underwent islet cell transfer from the left upper quadrant to the right lower quadrant (Stage III). One dog failed to become diabetic at this time and was sacrificed, feeling that some pancreatic tissue had been left in the peritoneal cavity. The other 4 dogs became diabetic immediately after the islet cells were transferred showing that the transplant had been the sole source of insulin production. They were then treated with insulin to tide them over until the islet cells could again grow and produce insulin.

Insulin could be withdrawn from 2 of these dogs, 30 days and 52 days

***Somagen (protein veal and liver concentrates) Upjohn Co., Kalamazoo, Michigan.

respectively following islet cell transfer. The first dog lived without insulin for 5 wks but expired from a fulminating bloody diarrhea before the final stage procedure could be performed. The other dog lived without insulin 8 wks and then underwent total excision of the transplant. He became rapidly diabetic and expired in diabetic coma on the 16th postoperative day.

The remaining 2 dogs have been controlled on decreasing amounts of insulin so that approximately 5 mo following islet cell transfer they require less than 5 units of NPH insulin daily. These dogs have not yet undergone the final stage operation.

SUMMARY

1. A series of staged operations was done in an effort to autotransplant functioning islet cell tissue.

2. The transplanted tissue was transferred to a new location on the abdominal wall and showed evidence of active function after a latent period of from 30 to 52 days.

3. Removal of the autograft after a period of survival without insulin again produced the diabetic state indicating that the graft had been the sole source of insulin.

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Obstetrics and Gynecology

THE PROLIFERATIVE CAPACITY *IN VITRO* OF TISSUE EXPLANTED FROM HISTOLOGICALLY BENIGN AND MALIGNANT AREAS OF THE SAME UTERINE CERVIX*

J G MOORE AND W W BRANDHAM

As previously reported¹ the observation was made that histologically normal epithelium explanted from a cervix containing cancer grew with increased proliferative vigor. It is the purpose of the present paper to substantiate the observations made earlier and increase our knowledge of the growth characteristics of the histologically benign areas of the cervix associated with nearby malignancies.

METHOD

Tissue obtained by cervical biopsy was divided into 2 portions: 1 for histological section and staining and the other for tissue culture studies. The latter portion was explanted aseptically as described in a previous publication² and a nutritive medium composed of placental cord serum 40 per cent, beef embryo extract 4 per cent, and a balanced salt solution (Hanks) 56 per cent added. Estimates of growth potential were made from the extent and rapidity of growth. Cellular morphology was documented by photomicrographs of the living tissue through the use of the phase microscope and by fixed and stained cultures (hematoxylin and phloxin).

Two areas of each cervix were biopsied. One biopsy taken from the suggestive area of the cervical lesion and the other from an expected normal area approximately 1 cm from the site of the lesion. The explanted tissue from each area was maintained identically in separate cultures. Of patients having no lesion or history of malignancy, tissue was explanted in a similar manner—one biopsy from the squamo-columnar junction and the other from a point 1 cm distant on the exocervix. Cervical biopsies were taken from 2 types of patients: 1) those having known cervical malignancies and 2) those having normal cervices.

RESULTS

In 12 cases of cervical carcinoma in whom comparative cultures of the benign and malignant areas of the same cervix were grown, 83 per cent of the cultures of the normal cervical epithelium showed a good/excellent proliferative capacity (Table 1). It is perhaps understandable that only 75 per cent of the malignant areas resulted in cultures showing a good/excellent growth response since the malignant areas were often necrotic and inflamed. The cells extending from the normal explant (Fig 1) showed a uniformity of structure and pattern of growth, whereas those growing from malign

*From the Department of Obstetrics & Gynecology, University of California School of Medicine, Los Angeles, Calif. Supported by Grant No. C2413, USPHS.

Table 1 Proliferative Capacity of Malignant (A) and Benign (B) Cervical Epithelium from the Same Cervix

PATIENT	DIAGNOSIS	AREA	PROLIFERATIVE CAPACITY
1	54 Squamous cell carcinoma	A	No growth
	54 Squamous cell carcinoma	B	Good
2	74 Squamous cell carcinoma	A	Excellent
	74 Squamous cell carcinoma	B	Good
3	134 Squamous cell carcinoma	A	Excellent
	134 Squamous cell carcinoma	B	Excellent
4	144 Squamous cell carcinoma	A	Good
	144 Squamous cell carcinoma	B	Excellent
5	164 Squamous cell carcinoma	A	Good
	164 Squamous cell carcinoma	B	Good
6	184 Minimal invasion	A	Excellent
	184 Minimal invasion	B	Excellent
7	26 Squamous cell carcinoma	A	Excellent
	26 Squamous cell carcinoma	B	Excellent
8	46 Squamous cell carcinoma (necrotic tissue)	A	No growth
	46 Squamous cell carcinoma (necrotic tissue)	B	No growth
9	76 Squamous cell carcinoma (contaminated 7 months)	A	No growth
	76 Squamous cell carcinoma (contaminated 7 months)	B	No growth
10	86 Squamous cell carcinoma 1360r X radiation	A	Good
	86 Squamous cell carcinoma	B	Excellent
11	96 Squamous cell carcinoma	A	Good
	96 Squamous cell carcinoma	B	Good
12	106 Squamous cell carcinoma	A	Excellent
	106 Squamous cell carcinoma	B	Excellent

nant explants (Fig 2) displayed marked cellular pleomorphism. Comparative photomicrographs of higher magnification (Fig 3) indicate further the varying cell types prevalent in the cultures of malignant tissue as contrasted with the uniformity of the cytoplasmic/nuclear ratio and staining characteristics of the normal autologous tissue. In contrast with cultures made from identical biopsies taken from 10 cervixes in which no malignancy could be demonstrated, only one showed even a minimal growth response (Table 2).

It is noteworthy that work now in progress shows that the malignant areas of questionably malignant cervical lesions (i.e. carcinoma *in situ* et cetera) grow in culture with equal vigor as frank cervical carcinoma. More

Table 2 Proliferative Capacity of Normal Adult Cervical Epithelium

PATIENT	DIAGNOSIS	PROLIFERATIVE CAPACITY
1	99 Normal Cervix	No Growth
2	83 Normal Cervix	No Growth
3	119 Normal Cervix	No Growth
4	193 Normal Cervix	No Growth
5	223 Normal Cervix	No Growth
6	273 Normal Cervix	No Growth
7	225 Normal Cervix	No Growth
8	335 Normal Cervix	No Growth
9	445 Normal Cervix	No Growth
10	16 Normal Cervix	Very Restricted Growth

Fig 1 (A) Stained histological section of normal area 80X (B) Stained tissue culture growth 80X

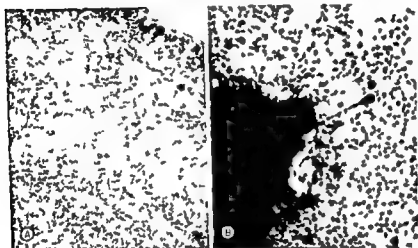
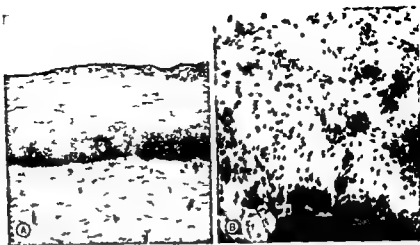
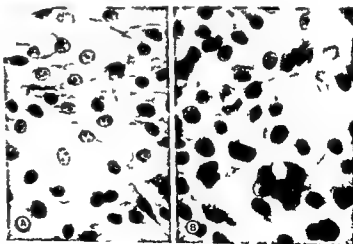


Fig 2 (A) Stained histological section of malignant area from same cervix shown in Fig 1A 80X (B) Stained tissue culture growth 80X

Fig 3 Comparative photomicrographs of higher magnification of Fig 1B and 2B 200X. Note pleomorphism and hyperchromatic cell of malignant area



over in 16 separate experiments so far histologically normal areas of these cervixes containing controversial lesions have shown the same vigorous response in 8.2 per cent of the cultures. Also it should be stated that pregnant cervical epithelium shows a definitely increased proliferative vigor. Of tissue cultured from 13 such cervixes 69 per cent resulted in a definite but uniform growth response. None demonstrated such a marked proliferative

capacity as was observed in cultures of malignant or questionably malignant cervical epithelium

DISCUSSION

The problem of what causes normal cervical epithelium when associated with malignancy to proliferate so vigorously is a most intriguing one. The growth potential of this tissue appears equal to that of malignancy, however its ability to continue at an extended high rate of growth is less marked. In these instances the histologic section of the normal cervical epithelium revealed no cytologic variation that might justifiably be termed a precancerous condition. Additional study concerning the factors contributing to this enhanced growth response of normal tissue associated with malignant tissue appears to be warranted.

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STILL AND MOTION PICTURE HYSTEROSALPINGOGRAPHY*

JOHN A. KERNER, EARL R. MILLER AND ALLAN PALMER

Hysterosalpingography has proved to be one of the most useful diagnostic techniques in gynecology. It has been used extensively in our infertility clinic at the University of California and in private practice. The technique is useful in demonstrating defects of the corpus and cervix of the uterus, abnormalities of the tubes and movement of the opaque material in the peritoneal cavity. In some cases it appears to be therapeutically beneficial. During the last 2 years motion picture studies of the hysterosalpingogram have been instituted making use of an image intensifier and this new work has been most rewarding.

METHOD

To facilitate performing a large number of hysterosalpingograms it was desirable to make our procedures as simple as possible. Of several types of apparatus used for injections the most suitable for our purposes was the Riaz Palmer instrument* which has been described by Raoul Palmer of France. This apparatus is compact and easy to handle (Fig. 1). Its features include a metal syringe with screw handle, an aneroid manometer which is fastened directly to the syringe and a simple stand. A properly made screw

*From the Department of Obstetrics and Gynecology and the Department of Radiology of the University of California School of Medicine, San Francisco. The image intensifier and camera used for these studies were made available for use through an institutional grant from the American Cancer Society.

*Manufactured by Collin et Cie, 6 Rue de l'Ecole de Medicine, Paris VI.

FIG. 1



tip cannula used gently and fit tightly is least painful for the patient. Such a cannula makes it possible to dispense with a speculum and provides a means by which one obtains excellent visualization of the endocervix and internal cervical os. Cannulas of the Colvin design are made in 3 sizes for our use by the instrument maker at the University of California hospitals.

It is customary to use the apparatus for making a series of films as an outpatient procedure and to follow the immediate study with a film made 24 hr later.

A variety of media has been used and we have come to the conclusion that for our purposes all currently available water soluble media absorb too rapidly. Because there is so much information obtained from the 24 hr film the examination should be carried out to at least this length. Accordingly a low viscosity form of lipiodol (Ethiodol)* was selected and this agent has been used over 1 000 times in some 600 patients. The medium is ideal for 24 hr films yet it is absorbed within 3 to 4 wks. The few febrile reactions noted early in the series have been eliminated by the use of antibiotic drugs prophylactically whenever there was suspicion of pelvic inflammatory disease or when more than 150 mm of mercury pressure was necessary. No other reactions of importance have been observed.

During the last 2 years the fluoroscopic image intensifier has been used as an adjunct to the hysterosalpingogram. This apparatus is described in detail elsewhere.^{2,3} Briefly, by means of an electron-optical system the image intensifier produces a fluoroscopic image several hundred times brighter than the original produced by x-ray. This makes it feasible to produce satisfactory motion pictures (Fig 2) of a fluoroscopic image and to view the image in subdued light. Exposure of both physician and patient to scattered x-ray is significantly reduced. A most interesting series of motion picture films has

*Supplied for this study by the E. Fougera & Co., Inc.

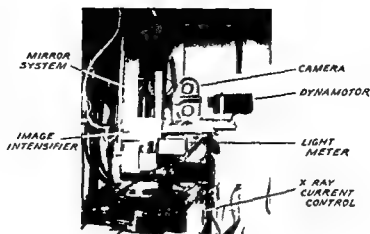


Fig. 2

resulted from the combined use of the image intensifier and the well established technique of Lithiodol injection

RESULTS

Findings with the Hysterosalpingogram The use of hysterosalpingography as a diagnostic procedure has been generally accepted. It reveals gross abnormalities of the uterine cavity and tubes. In addition the technique often reveals crypts in the endocervix which may represent unexpected foci of infection. It may show abnormalities of the internal os not clearly disclosed in any other fashion. The 24 hr film gives a clue to abnormalities within the pelvic peritoneal cavity.

Findings with the Motion Picture and Image Intensifier Although fluoroscopic studies have always been of interest in hysterosalpingography, it has been impossible to re-examine any part of the procedure except by occasional spot films. The motion picture has provided us and other workers³ with a permanent record of the fluoroscopic study. The motion picture has revealed intrauterine pathology which was often missed in the standard film taken when an entire defect or mass was obscured by the medium. The motion picture has shown the radiopaque medium during the time it was surrounding a mass or flowing into a defect. It also has presented, in a dynamic fashion, the physiology of the tube and peritoneal cavity.

The Philips Image Intensifier, used so far, has a relatively small field and the individual frames of motion picture film do not compare in definition with the spot films. However, it is felt that newer equipment will provide not only a larger field but also clearer pictures.

SUMMARY

A technique of hysterosalpingography and motion pictures in hysterosalpingography has been briefly described. The hysterosalpingogram has proven to be invaluable in the study of certain infertile women and as a diagnostic aid in other gynecologic disorders. The motion picture made by using the image intensifier becomes a new tool which adds to our understanding of the physiology of the uterus, tubes and peritoneal cavity and may become of increasing usefulness in the more routine studies of the pelvic organs.

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CLOSURE OF THE INCOMPETENT CERVIX DURING PREGNANCY*

ROBERT H. BARTER, JAMES A. DUSARBEK, H. L. RIVA
AND JOHN PARAS

That the cervix may be inadequate to contain the enlarging products of conception within the uterus has been suggested simultaneously by two gynecologists working in different parts of the world—namely Ish in Chicago and Shirodkar in India. Ish proposed a plastic operation to correct cervical incompetency and first published his results in 1950¹ although his work dated back to 1911. Shirodkar's purse string procedure did not become known in this country until after his presentation at the International Congress on Gynecology Obstetrics in Geneva, Switzerland in 1954.²

The concept of midpregnancy loss due to cervical incompetence has not been accepted by all obstetricians and gynecologists. However, there is a small but definite group of patients who have habitual abortion or immature labor after the 16th week of gestation. The usual sequence of events is that of painless effacement and dilatation of the cervix with subsequent prolapse and rupture of the membranes. The latter is followed by abortion or the delivery of a pre-viable infant.

The diagnosis of this condition may be made by radiological means³ by weekly or biweekly examination of the cervix during a pregnancy in which the cervix manifests incompetency or from the history of repeated late abortions or immature labors always preceded by rupture of the membranes.

It is with some reluctance that this new procedure is being reported for the first time in the United States for our results have not been as good as those reported by Shirodkar. However, with improvement in technique better results are anticipated. There has been no desire on the part of the authors to report this procedure without serious forethought.⁴

MATERIAL AND RESULTS

The patients in this series are from the obstetric services at the George Washington University Hospital, Walter Reed Army Medical Center, D.C.

From the Departments of Obstetrics and Gynecology, The George Washington University School of Medicine, Walter Reed Army Medical Center and D.C. General Hospital.

General Hospital and 1 patient from the Alexandria Hospital Alexandria Virginia * The patients surveyed in this report have been treated during the past 21 months. During that time approximately 16 250 patients have been delivered at the first 3 hospitals named. It is evident that in any carefully controlled series the number of patients with habitual abortions due to an incompetent cervix will be extremely small. The latter point is worthy of repeated emphasis. Fascial closure of the cervix to prevent other types of abortion will be uniformly unsuccessful.

Sixteen patients are reported at this time. These patients have been divided into 2 groups: those in whom fascial closure of the cervix was accomplished either with a successful or an unsuccessful outcome, and those in whom fascial closure of the cervix has been done during a pregnancy which is still in progress.

There were 11 patients in the first group. Those 11 patients had had a total of 57 pregnancies including those pregnancies in which closure of the cervix was accomplished. Thirty eight of the previous gestations had ended in abortion. This group of patients had only 8 living children as a result of their first 46 gestations, which represents a salvage rate of 17.4 per cent. Of the 11 patients operated upon 5 had surviving infants for a survival rate of 45.4 per cent, a significant increase over the previous figure of 17.4 per cent. Four of the 5 surviving infants were premature babies but all have done well.

In the second group there are 5 patients. These 5 patients have had a total of 27 pregnancies, 20 of which had resulted in late abortions or the delivery of pre-viable infants. Only 2 infants have survived as the result of the first 22 gestations in this group. These 5 patients at the present time are in varying stages of late pregnancy and it would appear that all have a fairly good chance of having surviving infants.

In both groups there have been a total of 84 gestations. Eliminating the 5 patients who are pregnant, there has been a total of 79 pregnancies with only 15 surviving infants including the 5 infants who survived after fascial closure of the cervix during pregnancy.

If one accepts the figure quoted by Malpas, namely that any habitual aborter has a 27 per cent chance of having a successful outcome in any given pregnancy, then the figure of 45.4 per cent successful outcome in the first 11 patients would appear to assume importance. That percentage figure will be improved when the 11 remaining patients have completed their pregnancies.

TECHNIQUE

The operation is best carried out between the 12th and 18th week of gestation or even later if there has not been too much effacement and dilatation of the cervix. The results in this series have been least satisfactory in those patients in whom the cervical dilatation was greater than 4 cm. at the time of operation.

With the upper vagina adequately exposed by means of retractors, the anterior and posterior lips of the cervix are grasped with ring forceps. If there is significant dilatation of the cervix the operation is best done with the patient in rather steep Trendelenburg position. An opening is made in the midline of the vaginal mucosa overlying the cervix anteriorly. This inci-

* Patient of Drs. George Speck and Paul Halter, Alexandria, Virginia.

sion is made as high as possible above the lower limits of the cervix and should be $1\frac{1}{2}$ " or 2" above the most dependent portion of the cervix. The incision is widened laterally so that it is $1\frac{1}{2}$ " long. The bladder is then dissected off the cervix and pushed as high as possible. The posterior portion of the vaginal mucous membrane is then exposed and a small incision made in the midline 2" or $2\frac{1}{2}$ " above the external portion of the cervix. A strip of fascia is then threaded to a large aneurysm needle (as designed by Shirodkar). The fascial needle is placed in the anterior incision and it is gently forced posteriorly around the right side of the cervix. The needle should emerge through the incision in the posterior wall of the vagina. The leading end of the fascial strip is pulled through the posterior incision and the aneurysm needle removed. A second aneurysm needle (the mirror image of the first) is then introduced anteriorly and gently forced along the left hand side of the cervix to emerge through the previous posterior incision. The fascial strip is tied to the aneurysm needle and the fascia pulled through to the anterior incision. The fascia is now in position to be drawn around the cervix in the form of a purse string. If the membranes are bulging they will recede into the uterus as the fascia is tightened. The fascial strip is pulled snugly and the two overlapping ends are sutured together with silk, cotton or linen and then anchored to the substance of the cervix. The anterior vaginal incision is closed. The fascia is then anchored to the cervix posteriorly by means of a nonabsorbable suture and the posterior vaginal mucosa is closed with a catgut suture. The patient is kept in bed for 10 to 14 days after which she is allowed to have a reasonable degree of activity.

Because it was impossible to run a control series in this small group of patients most of these patients have received adjunctive therapy in the form of vitamin C, progesterone and in the Walter Reed patients injections of *Distrium*.

DISCUSSION

Closure of the incompetent cervix during pregnancy appears to have a very definite place in an extremely small group of patients who have had repeated painless effacement and dilatation of the cervix after the 16th week of gestation. Iashi has noted that many of these patients have had some type of trauma to the cervix. These include forcible dilatation of the cervix during a curettage, cervical lacerations from a precipitous labor, a previous traumatic forceps delivery, Durhssen's incisions or a vaginal hysterotomy. The patient should give a typical history of repeated late pregnancy loss which has been initiated by spontaneous rupture of the membranes without antecedent pain. In the patient with this condition the effacement and dilatation of the cervix with protrusion of the membranes may have been observed in a previous pregnancy.

The particular advantage of this operation over the Iashi procedure is that it can be accomplished during pregnancy. The principal disadvantage of the Iashi operation is that it has to be done in the postabortal state or during a nonpregnant interval. Therefore this procedure has the advantage of being capable of preserving a pregnancy.

The alternative to surgical closure of the cervix in this group of patients is complete bed rest during the last 4 or 5 mo. of gestation. Complete bed rest may be accepted by the patient during one gestation but it will be

accepted rarely in a subsequent gestation. Thus a very real advantage of the surgical approach is that the patient may remain ambulatory following the procedure.

The fascial used can be homologous (taken from the patient's own thigh) or preserved ox fascial can be used. At the present time our preference is for the homologous fascial. The use of substances such as polyethylene also has been reported.¹¹

The patient should be delivered by cesarean section after this procedure to preserve the newly established cervical competency. Shirodkar has reported that he has delivered 21 infants from the 19 patients in whom he initially did the procedure. In the event that the patient does not desire more children she could be delivered from below. However most of these patients with their previous high pregnancy wastage are desirous of having more than one baby; thus cesarean section is usually the delivery method of choice.

Due to the fact that this method of treatment subjects the patient to not 1 but 2 operative procedures it is again emphasized that it should be undertaken under only the most exacting indications. This operation has no place in the treatment of repeated early abortions and it is no panacea for the treatment of all late abortions.

SUMMARY

1 A new procedure for closure of the cervix during pregnancy in the presence of an incompetent cervix has been introduced into this country.

2 The procedure was initially developed by Dr V. N. Shirodkar of Bombay, India.

3 The operation has been successful in 45.4 per cent of a small series of patients operated upon to date.

4 An additional 5 patients who have had the operation are still pregnant and should add to the ultimate success of the operation.

5 This operation predisposes the patient to cesarean section.

6 This surgery for the correction of habitual late abortions or immature labors is applicable to only a very small, highly select group of patients.

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THE EFFECT OF POSITION ON THE COURSE OF THE SECOND STAGE OF LABOR*

MICHAEL NEWTON

Most physicians in this country are accustomed to conducting the second stage of labor with the mother in the lithotomy position. Its main advantage is that when used in conjunction with the equipment of the modern delivery room it is easier to ensure asepsis to handle obstetrical hemorrhage to care for the newborn and to terminate the second stage of labor, when necessary, by means of forceps and episiotomy.

However looked at from the perspective of history, this position is simply a newangled and sitting kneeling squatting or other positions have been used for countless generations. Soranus Gynecology is recently translated by Dr. Oswei Temkin contains a section on the construction and use of a midwife's stool or obstetrical chair as was employed in the second century A.D. In the middle ages also the obstetrical chair was an important adjunct to the practice of midwifery. Even today many primitive women continue to deliver their babies in the sitting squatting or other positions. We have seen this at the University Hospital in Mississippi in women who have delivered several previous babies in their own cabins. Some of them want to kneel on the floor and we have difficulty in persuading them that the delivery table is the proper place.

There can be no doubt that modern methods of obstetrical care have increased the safety of both mother and child. However in discarding age old positions for the second stage of labor have we adopted a technique which is simply more convenient for the mother's attendants but less mechanically efficient and comfortable for the mother herself?

In considering this question 3 problems present themselves. First which position is more efficient for the expulsion of the baby? Secondly if the sitting or squatting position is more efficient can it be adapted to the demands of a modern delivery room? Thirdly does the use of this position improve the management of the second and third stages of labor? The present study represents the preliminary phases of an attempt to answer these questions.

During the second stage of labor the mother's voluntary muscles of expulsion are used to augment the uterine contractions in pushing the baby down the lower birth canal. The muscles consist of the abdominal muscles the diaphragm and the accessory straining muscles. In the sitting position when the back is curved the force of these voluntary muscles is directed towards the pelvis. In the supine position with the feet unsupported part of their force is dissipated in pushing the abdominal wall forward. An important corollary of this is that when the expulsive muscles contract less efficiently reciprocal relaxation of the muscles of the pelvic floor is also less efficient. In addition gravity in the form of the abdominal contents and the weight of the baby is effective in the sitting or squatting position but not in the lithotomy position.

The same expulsive muscles are used though to a lesser degree in the everyday functions of defecation and urination. These functions are com-

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accepted rarely in a subsequent gestation. Thus a very real advantage of the surgical approach is that the patient may remain ambulatory following the procedure.

The fascia used can be homologous (taken from the patient's own thigh) or preserved ox fascia can be used. At the present time our preference is for the homologous fascia. The use of substances such as polyethylene also has been reported.⁶

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6. Bryant, Richard. Personal communication.

There is one more important point to note about the position. The head is bent forward by a pillow. This helps to put the whole body in a curved line and increases the efficiency of the expulsive effort. During contractions the woman should be encouraged to flex her head still further.

The modified sitting position is produced by the back rest. It offers no difficulty in the use of procedures such as cleansing of the perineum, control of delivery, episiotomy, or the application of forceps. At first it was thought that anesthesia might represent a problem. It does so only if deep general anesthesia is required; then the supine position is of great help to the anesthesiologist. However, the vast majority of normal obstetrical patients do not require deep anesthesia and when deep anesthesia is necessary the back rest can be put down quickly and easily.

Evaluation of the effect of the semi-sitting position with the back curved on the second stage of labor represents a difficult problem, since many factors enter into it. At the present time we have data on the use of the back rest in 86 patients. These patients form part of a series of 220 patients followed consecutively at the University Hospital between February and May of 1956. Various physical and psychological data were recorded for each of these patients and a large number of them were interviewed in the immediate postpartum period. The details of the experimental method will be published elsewhere. Suffice it to say that the data sheets were constructed so that they could be placed on IBM cards for statistical evaluation. The sheets were filled in during or immediately after labor by the person who had had most contact with the woman during her labor and delivery, i.e. by the student or intern.

Records of the labors of these 86 women seem to us to indicate that the use of the back rest is entirely compatible with convenient and comfortable labor.

Most of the women progressed through the second stage of labor with little need for anesthesia. Only 14 per cent had caudal spinal or nitrous oxide anesthesia. Twenty-six per cent found that they needed whiffs of Tri-lene. More than half (60 per cent in fact) had no anesthesia at all or merely local infiltration or pudendal block. The result of this lack of anesthesia was that only 3 per cent of the women were unconscious or deeply depressed at the time of delivery.

The great majority of women were self-controlled and cooperative during delivery. Only 7 per cent did not cooperate with requests and directions and only 8 per cent screamed. The others responded to directions by the attendants part or all of the time and were either quiet or made moderate vocalizations.

The reaction to the baby was mostly a pleasant one. Only 8 per cent of the mothers were indifferent. None showed active disgust when they first saw the baby. The majority smiled a little, but 35 per cent were greatly pleased.

The obstetrical details of the delivery were similarly normal. Forceps were used in less than a quarter of the cases. In only 2 per cent were mid-forceps necessary.

Injury to the perineum was also low. Although episiotomies were not performed on 62 per cent of the patients, only 6 per cent had second-degree tears and there were no third-degree tears. The third stage of labor was

monly and easily performed in the sitting or squatting position with the back curved. The greater efficiency of these positions will be apparent to anyone who has ever been compelled to make a bowel movement lying flat on a bed pan.

Thus it is reasonable to suppose that childbirth like urination and defecation is more efficiently accomplished sitting or squatting with the back curved.

The second problem is that of using these mechanical advantages in such a way that the ease of dealing with obstetrical problems is retained. The use of the traditional obstetrical chair would make it extremely difficult to secure ischias to control hemorrhage or to use obstetrical forceps.

One solution to this problem has been done recently is to raise the obstetrical chair in the air and thus permit the attendant to work under his patient.

A simpler method is to fit a back rest to the conventional delivery table. Such a back rest has been constructed for use in our department. It was made from inexpensive materials by the shop mechanic in our Department of Pharmacology. It is somewhat modified from a similar back rest used at the O'Connor and Franklin Hospitals in San Francisco. The idea for it came from Mrs. Mabel L. Fitzhugh, a physical therapist, who has been greatly interested in education for childbirth in the San Francisco area for the past several years and who is working as a research associate in our department.

Figure 1 shows the Fitzhugh back rest in action. The curve of the mother's back should be noted. The angle of the back rest can be adjusted. Up to the present time we have used an angle of 20 to 40 degrees but this may not be enough and we plan to increase this angle in future studies.

Until the head begins to show at the introitus the legs are allowed to be free. In this way the patient can most effectively bear down by pulling on her own legs with her hands just below the knees.

When the stirrups are placed under the legs they are lowered as far as possible and care is taken to avoid separating the legs too widely. The foot plate is adjusted so that the mother can push hard with her feet but has no pressure under the knees. She can help herself by pulling on the handgrips. Whenever possible we prefer not to restrain the hands.

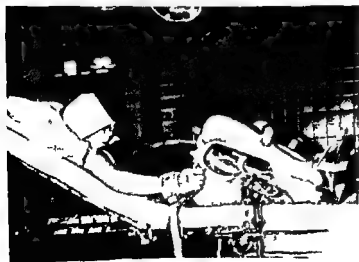


Fig. 1 Position on delivery table in 2nd stage of labor using Fitzhugh back rest

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rapid with only 5 per cent having retention of the placenta for more than 20 min

These records are quite similar to the other deliveries which took place in the University Hospital at this time. There is nothing to suggest that the back rest has in adverse effect on the second or third stages of labor.

The data so far available indicate that no disadvantages are apparent from the use of the back rest. A number of very favorable clinical impressions have come out of this investigation. These are:

1 Increased comfort to the woman in the second stage. This is particularly noticeable when a patient is raised from the lithotomy to the propped position.

2 Increased effectiveness of expulsion. This has been noted on a number of occasions when the back rest was raised in women who were making ineffective efforts during the second stage.

3 Increased ability of the patient to see and hold her baby immediately after the delivery.

4 Increased ability of the woman who has been educated for childbirth to cooperate since she is not lying flat and out of the picture.

Because our records show that there appears to be no adverse effects and because of our favorable clinical impression, we plan to investigate the problem further.

We plan during the coming year to do a true experimental study on the second stage of labor using comparable test and control groups.

SUMMARY

1 The greater efficiency of the propped position with the back curved for the expulsive effort has been discussed with particular reference to the second stage of labor.

2 An adjustable back rest has been used for the conduct of the second stage of labor.

3 The use of the Fitzhugh back rest is quite compatible with the best methods of modern obstetrical care.

4 A preliminary study of the effects of conducting the second stage of labor in the propped position with the back curved has been carried out in 86 women.

5 Clinical impressions from this study indicate increased comfort to the mother, greater efficiency of the expulsive efforts and considerable advantages in a program of cooperative childbirth.

POSTMATURITY AND FETAL MORBIDITY

EDWARD H. BISHOP

The subject of postmaturity remains an obstetric enigma in spite of many scientific and clinical investigations. When these studies are reviewed they present varying results with controversial and diversified conclusions. Two essential points remain unanswered. The first is whether or not prolongation of pregnancy actually exists as a pathological state. Even when its existence is conceded a second problem still remains that of management. The recommended managements vary from a hands off and wait approach of some to suggestions of others for elective termination of pregnancy. This latter attitude is typified by the majority of English writers on this subject. The pediatric observations of Clifford¹ and the fetal blood oxygen saturation studies of Walker² have offered clinical and scientific support to those who advocate interference in order to avoid the supposed hazards of postmaturity. Unfortunately these investigations have not answered the problem of the obstetrician. He cannot observe the baby before its birth and there are no means by which he may determine prenatally the amount of oxygen being delivered to the infant. Instead the clinician must make the diagnosis of postmaturity only by the inexact signs and symptoms which are classically used for determination of the expected date of delivery. Probably the most reliable of these is the menstrual history but even this is renowned for its physiologic and pathologic variations. The clinician must also as a result of his experience or that of others decide which of the methods of management offers the lesser fetal risk that of waiting for the spontaneous onset of labor or that of interference. This clinical study is presented as a step towards solving these problems of the clinician.

All patients both ward and private delivered at the Pennsylvania Methodist and Lankenau Hospitals during a period of 1 yr were used as the material for this study. These hospitals vary sufficiently in their geographic location, type of patient and social group served to offer a cross section and an average sampling of the general population. When each patient was registered for delivery at a hospital the expected date of delivery was noted on the hospital records. If a patient was delivered more than 14 days after the recorded expected date of delivery she was interviewed personally by the author or by an obstetric resident who had been assigned to this problem at each hospital. There were 305 patients who comprised this group of supposedly postmature deliveries. However the personal interview revealed that in 118 instances there had been enough previous variation in the menstrual history or enough question regarding the exact time of onset of the last menstrual period to make the diagnosis of postmaturity equivocal. These doubtful cases were discarded and this study is concerned with the remaining 187 cases who were considered as definitely postmature from the clinical point of view. The incidences of premature delivery, mature delivery and postmature delivery are shown in Table 1. The incidence of 3.2 per cent postmaturity is significantly less than that reported in many series using the same definition of delivery 294 days or more after the first day of the last menstrual period. This decreased incidence is probably

Table 1 Incidence of Postmaturity

	NO	%
Immature deliveries	109	7.1
Mature deliveries	5,164	89.7
Postmature deliveries	187	3.2

Table 2 Method of Delivery

	ALL CASES		POSTMATURE	
	NO	%	NO	%
Spontaneous	2370	11.1	61	32.6
Low Forceps	2728	47.4	94	50.3
Mid forceps	142	2.5	15	8.0
Breech	178	3.1	7	3.7
Version	6	0.1	0	0.0
Cesarean	336	5.8	10	5.4
Total	5760	100.0	187	100.0

Table 3 Perinatal Mortality

	PREMATURE	MATURE	POSTMATURE
Stillbirths	11.0%	0.9%	2.1%
Neonatal	22.3%	0.3%	2.1%
Total	33.3%	1.2%	4.2%

a result of having obtained the information directly from the patient rather than from her record alone.

A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2. The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8.3 per cent in the term group and 13.4 per cent in the postmature group. This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicated a longer labor and more difficult delivery. The average weight of the postmature baby was 7.7 lbs. and 44 per cent of all postmature babies weighed 8 lbs. or more. This seems adequate reason for the moderate increase in major operative obstetrics.

The primary factor which has aroused the concern of many regarding postmaturity is the reported increase in the fetal death rate in this group. It is the number of unexplained fetal deaths which prompt many writers to advise termination of pregnancy. The perinatal mortality of all categories—premature, term and postmature—in this series is shown in Table 3. The perinatal mortality of 4.2 per cent in the postmature group is significantly higher than that of the mature group and is equally divided between stillbirths and neonatal deaths. It is the comparable figures of other authors which have led to the conclusion that pregnancy should be terminated if it continues more than 14 days after the calculated date of delivery. However, it is necessary to examine the causes of fetal death before it is possible to reach a similar conclusion from our experience. These causes as

determined by both clinical observation and autopsy examination were as follows 1) Erythroblastosis 2) Anoxemia associated with difficult delivery 3) Diaphragmatic hernia and bilateral bronchopneumonia 4) Intracranial hemorrhage following difficult delivery 5) Congenital aplasia of the adrenals 6) Bilateral diaphragmatic hernia 7) Unknown Macerated stillborn 8) Anencephalic monster

In this series congenital lesions were responsible for the deaths of 4 babies birth trauma for 2 and erythroblastosis for 1 It is unfair to compare the corrected incidence of 1 death in the postmature group with the uncorrected deaths in the mature group but it can be stated without equivocation that earlier termination of pregnancy would not and could not have salvaged those babies dying of congenital lesions It is equally true that only better obstetric judgment rather than termination of pregnancy would have salvaged the 2 that died as a result of birth trauma In direct contrast one hesitates to estimate what the fetal loss might have been if all of these 187 pregnancies of 294 or more days duration might have been terminated either by induction of labor under unfavorable circumstances or by cesarean section a procedure which carries with it an increased fetal loss when compared to vaginal delivery Clifford¹ has reported that among primigravida patients 26 yr or older who deliver after 300 days 1 out of every 3 babies die either in utero or after birth There were 42 patients in this series who fulfilled these qualifications and in this group there were 2 baby deaths rather than the prophesied 14 These 2 deaths were the result of diaphragmatic hernia and intracranial hemorrhage

CONCLUSIONS

1 The incidence of postmaturity in this series of 5760 deliveries is 3.2 per cent

2 The average birth weight of the postmature baby is slightly above average birth weight of the mature baby

3 There is an increased incidence of operative obstetrics in the postmature group probably related to the increased birth weight

4 Careful examination of the causes of fetal death in the postmature group does not reveal any constant hazard due to prolongation of pregnancy

5 There does not appear to be any justification for termination of pregnancy because of postmaturity However the chances of increased baby size must be constantly considered and appropriate obstetric management elected

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A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2. The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8.3 per cent in the term group and 13.4 per cent in the postmature group. This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicated on longer labor and more difficult delivery. The average weight of the postmature baby was 7.7 lbs. and 41 per cent of all postmature babies weighed 8 lbs. or more. This seems adequate reason for the moderate increase in major operative obstetrics.

The primary factor which has aroused the concern of many regarding postmaturity is the reported increase in the fetal death rate in this group. It is the number of unexplained fetal deaths which prompt many writers to advise termination of pregnancy. The perinatal mortality of all categories premature, term and postmature in this series is shown in Table 3. The perinatal mortality of 4.2 per cent in the postmature group is significantly higher than that of the mature group and is equally divided between stillbirths and neonatal deaths. It is the comparable figures of other authors which have led to the conclusion that pregnancy should be terminated if it continues more than 14 days after the calculated date of delivery. However, it is necessary to examine the causes of fetal death before it is possible to reach a similar conclusion from our experience. These causes as

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Table 1 Incidence of Postmaturity

	NO	%
Immature deliveries	109	7.1
Mature deliveries	5,161	89.7
Postmature deliveries	187	3.2

Table 2 Method of Delivery

	ALL CASES		POSTMATURE	
	NO	%	NO	%
Spontaneous	2370	41.1	61	32.6
Low Forceps	2728	47.4	91	50.3
Mid forceps	112	2.5	15	8.0
Breech	178	3.1	7	3.7
Version	6	0.1	0	0.0
Cesarean	336	5.8	10	5.4
Total	5760	100.0	187	100.0

Table 3 Perinatal Mortality

	PREMATURE	MATURE	POSTMATURE
Stillbirths	11.0%	0.9%	2.1%
Neonatal	22.3%	0.3%	2.1%
Total	33.3%	1.2%	4.2%

a result of having obtained the information directly from the patient rather than from her record alone.

A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2. The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8.3 per cent in the term group and 13.4 per cent in the postmature group. This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicting a longer labor and more difficult delivery. The average weight of the postmature baby was 7.7 lbs. and 41 per cent of all postmature babies weighed 8 lbs. or more. This seems adequate reason for the moderate increase in major operative obstetrics.

The primary factor which has aroused the concern of many regarding postmaturity is the reported increase in the fetal death rate in this group. It is the number of unexplained fetal deaths which prompt many writers to advise termination of pregnancy. The perinatal mortality of all categories—premature, term and postmature—in this series is shown in Table 3. The perinatal mortality of 4.2 per cent in the postmature group is significantly higher than that of the mature group and is equally divided between stillbirths and neonatal deaths. It is the comparable figures of other authors which have led to the conclusion that pregnancy should be terminated if it continues more than 11 days after the calculated date of delivery. However, it is necessary to examine the causes of fetal death before it is possible to reach a similar conclusion from our experience. These causes as

Collections from the football bladder receptacle were made once or twice daily measured and samples saved. The abdomen was inspected for dryness and the bag carefully inspected for leaks. If a leak was found the output of acid was not computed though the values for pepsin activity were determined.

To verify the functional state of the vagal innervation of the pouch insulin tests were performed on each animal. The volume of gastric juice was measured for two 30 min control periods after which the dog was given 5 or more units of crystalline insulin intravenously. The volume output was again measured at 30 min intervals for 2 hr. During the second or third interval after injection the output nearly doubled if vagus function was intact.

After 30 or more daily observations were recorded the neurosurgical operation was performed. Resections of portions of the cerebral cortex which are presumed to have connections with the hypothalamus and thus may initiate or control vagal impulses were made.

Thirteen intracranial operations were performed on 11 dogs. Bilateral prefrontal lobotomies were carried out on 3 animals and on one of these amputation of the frontal poles was ultimately done. Bilateral cingulate gyrus resection was done twice. Left cingulate gyrus resection was done twice and on one of these dogs the corresponding operation on the right side was later completed. Pituitary resection, left occipital lobe resection and right insular resection were each performed on one dog.

At autopsy the innervation of the pouch was established, the mucosa of the pouch and of the esophagoduodenostomy examined, the brain and the pouch preserved and appropriate microscopic sections were taken for study.

The daily volume of gastric juice was measured, the amount of free and total hydrochloric acid determined by titration with 11/N sodium hydroxide and the peptic activity analyzed by an egg white (digestion turbidometric method). The product of the volume in liters and the free acid in clinical units is the daily output of acid in milliequivalents. In the pepsin analysis the change in turbidity of a standardized egg white solution was measured after a period of digestion at uniform temperature by a sample of gastric juice.

The relationships of egg white concentration, time of digestion and pepsin concentration are described by the equation $dC/dt = k \times C$ where C is the relative concentration of protein at time t , k represents the velocity constant proportional to the rate of digestion of egg white and is proportional to the pepsin concentration. The value k is recorded in our records as pepsin activity. It has been computed from the formula $k = 1/(t_0 - t_1) \times \log C_1/C_2 = 0.46 \log C_1/C_2$. C_1 and C_2 the concentrations of the egg white substrate at the beginning and end of the 5 min digestion period are read from the standardization curve of the photolometer.

RESULTS

Table 1 lists the 19 surgical procedures performed on 11 total gastric pouch dogs, the control values for acid and pepsin, the postoperative values after each procedure for prolonged periods, the postoperative values in the first 10 to 14 days after each procedure, the number of measurements

Neurosurgery and Neurophysiology

THE EFFECT OF CORTICAL AND SUBCORTICAL BRAIN LESIONS UPON GASTRIC SECRETION IN DOGS WITH A VAGUS PRESERVED TOTAL GASTRIC POUCH*

HARRY M. RICHTER, JR., RICHARD A. DAVIS, DANIEL RUFF AND
NORMAN T. WAITER

The purpose of this study has been to investigate the possible influence of cerebral cortical and subcortical experimental lesions upon gastric secretion in the dog.

METHOD

Total gastric pouches were constructed in healthy mongrel dogs of 15 to 25 kg according to the technique outlined by Dragstedt.¹ Through a midline upper abdominal incision the pylorus and proximal duodenum were mobilized, freed of blood vessels for a length of 3 cm, and the duodenum was transected near the pylorus. The pyloric end of the stomach was closed and inverted. The stomach was pulled firmly down from the diaphragm, tensing both vagi and making them readily palpable on and behind the lower esophagus. The circumference of the stomach was dissected 1 cm below the esophagus deep to the peritoneum and the vagus nerves.

The only technical difficulty with the preparation occurred at the dorsal half of this dissection. If the dissection is conducted at the esophago-gastric junction instead of the lower level just described, one opens the chest and creates a hernia which is difficult to close permanently. The lower esophagus was grasped in a noncrushing clamp and the cardiac end of the stomach transected in the mobilized portion. The cardiac end of the stomach was closed and inverted, the esophageal end usually bearing 1 cm of gastric mucosa was anastomosed to the open bulb of the duodenum. A new strainless steel cannula was inserted into the gastric pouch, sealed with a turn of omentum and brought through a small incision in the abdominal wall to the left of the principal wound. The cannula was secured in its tight and dry track with metal and rubber washers and a ring fitted with a lock screw.

During the first postoperative week the animals were supported with daily subcutaneous injections of 500 cc or more of 0.9 per cent saline solutions and given prophylactic antibiotics. On the fifth day water was allowed and during the second week milk and ground meat were added to the diet. At the end of this period if alimentation was not adequate gavage feedings were given. If the blood loss was unusually great at operation 100 to 200 cc of whole blood was transfused. If food or hair was found in the stomach they were removed to prevent blockage of the cannula and perforation of the pouch.

*From the Department of Surgery, Northwestern University Medical School. Supported by U.S. Public Health Grant number H 920 (C).

contributing to each average the percentage rise or fall of acid and pepsin after each operation and the statistical significance of each change

In the table the dogs are grouped according to the type of neurosurgical or other operation performed. When a dog was subjected to more than one operation a new control series of observations was made before the later operation. Hydrochloric acid output is recorded as mEq/day (24 hr) and pepsin concentration as peptic activity from the computations noted above.

The study of the first 10 measurements of acid and pepsin during the first 2 postoperative weeks after the neurosurgical procedure was chosen to record the maximal response to stress and to allow for some days lost for record when collection bags were torn.

The results listed below as significant are statistically so to the degree indicated in the table. The biological meaning of this statistical significance has not been determined.

Two dogs were subjected to left cingulate gyrus resection. In the acute phase one had an increase in acid output; the other showed no change. In the chronic phase both showed a significant increase in acid. Decreases in pepsin appeared in both acute and chronic periods.

Of 3 dogs which had bilateral cingulus resections, all showed both early and prolonged decreases in acid output. In the acute period pepsin output was slightly decreased in 2 and markedly and significantly increased in the third. In the long term effect the pepsin was slightly increased in 2 and markedly increased in the third.

Of 2 dogs subjected to bilateral prefrontal lobotomy, early acid studies were made in 1 and showed a marked significant decrease. The long term effect was a very slight drop in both dogs. No significant change was observed in pepsin in the 1 dog studied.

One dog which had a left prefrontal lobectomy showed a slight early fall in acid but a long term rise. There was no early change in pepsin and chronic pepsin studies were not done in this dog.

The dog subjected to bilateral prefrontal lobectomy showed a slight early fall in acid but significant final rise. The early decrease in pepsin was not maintained.

Two dogs which had left occipital lobectomies showed no significant change in acid values. One showed a marked fall and 1 a marked rise in pepsin output.

Right insula resection in 1 dog produced a marked early fall in acid but no long term effect. Conversely, there was no early change in pepsin but after 2 wks a sustained rise.

Hypophysectomy caused no change in acid output but produced a prompt and sustained significant fall in pepsin.

Four dogs which had replacement of a broken cannula showed an early slight fall in acid output and an early marked fall in pepsin.

Vagotomy in 2 dogs following earlier neurosurgical studies showed an immediate marked and sustained fall in both acid and pepsin values.

SUMMARY

Neurosurgical procedures were performed in dogs in which total gastric vagus preserved pouches had been prepared.

Prefrontal lobotomies, prefrontal lobectomies, unilateral and bilateral

Table 1 Gastric Secretory Data*

DOC	TYPE OF OPERATION	CONTROL HCl	ACUTE CHANGE HCl	%	P	CHRONIC HCl	(n)	CHANGE %	P	CONTROL HCl	(n)	ACUTE CHANGE PFSN	%	P	CHRONIC PFSN	(n)	CHANGE %	P
34	Left singular resection	58	63.6	+24	> 0.3	62.7	(69)	+18	< 0.3	0.034	(22)	0.037	-31	> 0.3	0.046	(123)	-15	> 0.3
56	Left singular resection	40.3	41	+1	> 0.3	50	(52)	+22	< 0.3									
16	Bilateral singular resection	55.3	52	-5	> 0.3	31.4	(41)	-40	< 0.1	0.163	(19)	0.141	-1	> 0.3	0.191	(87)	+16	> 0.3
27	Bilateral singular resection	66.1	4.4	-28	< 0.1	61.7	(79)	-7	> 0.3	0.139	(34)	0.150	-6	> 0.3	0.173	(81)	+10	> 0.3
34	Bilateral singular resection	62.7	3.8	-46	< 0.1	41.7	(82)	-33	< 0.1	0.046	(123)	0.090	+71	< 0.1	0.072	(83)	+56	< 0.1
23	Bilateral prefrontal lobotomy	50.4	27	-47	< 0.1	47.6	(91)	-6	> 0.3	0.136	(43)	0.166	+6	> 0.3	0.171	(82)	+10	> 0.3
26	Bilateral prefrontal lobotomy	60.2				55.2	(31)	-9	> 0.3			0.140			0.142	(49)		
23	Left prefrontal lobotomy	47.6	42	-12		69.8	(98)	+45	< 0.1	0.171	(82)	0.173	+1	> 0.3				
24	Bilateral prefrontal lobotomy	70.2	63.4	-9	> 0.3	83.7	(31)	+27	< 0.3	0.134	(27)	0.123	-19	< 0.1	0.143	(42)	-6	> 0.3
39	Left occipital lobectomy	30.4	37	+22	(3)	31.2	(23)	+13	> 0.3	0.093	(61)	0.081	-10	(3)	0.077	(53)	-17	< 0.3
44	Left occipital lobectomy	19.4	96.6	-5	> 0.3	103.6	(32)	0		0.144	(61)	0.181	+26	< 0.3	0.182	(33)	+26	< 0.1
47	Right insula resection	36	23.6	-34		33.5	(63)	-7	> 0.3	0.144	(71)	0.151	+4	> 0.3	0.168	(33)	+17	< 0.1
32	Littorary resection	63.2	63	0		66.3	(80)	+3	> 0.3	0.224	(66)	0.112	-30	< 0.1	0.114	(101)	-50	< 0.1
25	Replace cannula	69.8	52.6	-23	< 0.1					0.173	(10)				0.156	(126)	-10	> 0.3
27	Replace cannula	83.3	22	-62	(5)					0.173	(81)	0.089	-49	(3)				
32	Replace cannula	64.4	39.4	-9	> 0.3	59.6	(17)	-9	> 0.3	0.224	(66)	0.170	-24	< 0.3				
34	Replace cannula	31.7	47.8	-8	> 0.3					0.074	(57)	0.048	-33	< 0.3				
25	Vagotomy	69.8	12	-83	< 0.1	11	(29)	-81	< 0.1	0.136	(126)	0.037	-70	< 0.1	0.044	(108)	-72	< 0.1
34	Vagotomy	41.7	24.9	-40	< 0.3	29.1	(80)	-10	< 0.1	0.072	(83)	0.023	-61	< 0.1	0.032	(11)	-55	< 0.1

*HCl as mEq/24 hr

Pepsin as peptic activity

Parentheses mark number of measurements

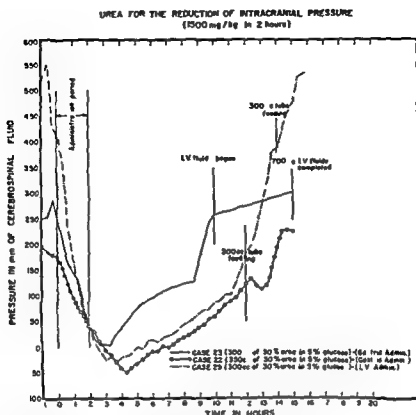


Fig 1 Effect of 1500 mg/kg of urea on cerebrospinal fluid pressure. In each case pressure fell to a level which was below normal even though there was a very high initial pressure in 1 of the patients.

patients for periods ranging from a few hours to 19 hr. In these studies data were also obtained on the following points: the effect of hypertonic sucrose and dextrose compared with urea; the relation of pressure changes to urinary output; the concentration of urea nitrogen in blood, urine, and cerebrospinal fluid at various intervals following injection.

The efficacy of urea for the reduction of intracranial pressure is illustrated in Figure 1. Here are represented the results on 3 patients with intracranial neoplasm, each of whom received 1500 mg/kg.

The study was conducted preoperatively in 1 patient (Case 23); it was carried out postoperatively in the other 2. The intravenous route was used in Case 29; the gastric route in the other 2 cases. All 3 patients were over 70 yr of age. A marked effect was seen in each instance. The pressure fell to a negative level, i.e., below atmospheric in 2 cases and to the zero level in the third. The pressure dropped promptly, as illustrated by the fact that the low level of approximately 40 mm was reached *before the 2 hr period of urea administration had been completed*. The effect persisted for about 12 hr in each case.

The 1500 mg/kg given in the cases just cited represents a large dose, and it should be noted that cerebrospinal fluid pressure fell to levels which were below normal even when there was a very high initial pressure. Since it has been a constant finding that response to a given dose of urea is greater when initial pressure levels are high, it is probably true that a dose

cingulate gyrus resections, left occipital pole resections, and an insular and pituitary resection were done. Of these operations only that of bilateral cingulate gyrus resection produced a consistent prolonged reduction in acid secretion. In fact it closely approximated the results obtained with vagotomy. This suggests that of the areas ablated the cingulate gyrus has the greater influence on the gastric acid production mediated via the vagus nerves.

In the early period where the stress of surgery played a role the alterations in acid output were generally downward. Likewise the initial pepsin determinations were usually decreased. It was noted that an increase in pepsin accompanied a very marked acid reduction.

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UREA IN THE MANAGEMENT OF INCREASED INTRACRANIAL PRESSURE*

MANUCHIR JAVID, PAUL SETTLAGE AND THOMAS MONTORE

Management of intracranial hypertension often presents a difficult problem to the neurosurgeon. Many agents have been used for the purpose of pressure reduction, none of which has proved to be ideal in all respects. The present study concerns the use of hypertonic solutions of urea, which we have been investigating for two years at the University of Wisconsin. To our knowledge there has been no other study of the effect of urea on intracranial pressure in man. A preliminary report¹ describing the early phases of the present study was published in March 1956.

To date urea has been administered to 66 patients from the neurosurgical service. The greater portion of these were cases of intracranial neoplasm. Included also were patients with head injury, hypertensive encephalopathy, brain abscess, hydrocephalus, meningitis, cerebrovascular accident and Meniere's disease. Doses ranging from 100 mg/kg to 1500 mg/kg were given both by the intravenous and oral routes. Intravenous urea has usually been given in 30 per cent solution with 5 per cent dextrose as a solvent. Cerebrospinal fluid pressure was measured in approximately half of the

*Division of Neurosurgery, Department of Surgery, Department of Anatomy, University of Wisconsin Medical School. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, Madison, Wisconsin.

fluid to escape from the lumbar puncture needle. It should be pointed out that the comparison of sucrose and urea was made on an equal volume rather than on equimolar basis. If one had wished to give an amount of 50 per cent sucrose which contained the same number of molecules as 256 cc of 30 per cent urea it would have been necessary to inject the obviously excessive and dangerous amount of 876 cc.

The evidence which has been collected thus demonstrates conclusively that cerebrospinal fluid pressure may be lowered effectively with the aid of urea. Single doses which are sufficiently large to bring initially elevated pressures down to negative levels are still within the range of safety and are well tolerated. The secondary rise in pressure which has been reported as occurring following administration of 50 per cent dextrose has not been a problem with urea.

Having satisfied ourselves as to the efficacy of urea as an agent for reducing intracranial tension we turned more recently to the problem of determining suitable schedules for prolonged administration. Our studies have shown that the drop in cerebrospinal fluid pressure is proportional to urine output and urea nitrogen excretion. This indicates that diuresis is the primary mechanism of action in producing the hypotensive effect. Water loss and dehydration cannot be maintained indefinitely so the problem resolves itself into one of determining what degree of dehydration spread over what period of time will be of greatest net benefit to the patient in terms of reduction of intracranial pressure. We know from experience that repeated doses of urea administered several times daily over a period of weeks continue to be effective. However since the effect lasts for a matter of 3 to 6 hr with moderate doses such a regimen would merely produce intermittent periods of pressure reduction. Is there any way in

UREA FOR THE REDUCTION OF INTRACRANIAL PRESSURE
- EFFECT OF DIFFERENT CONCENTRATIONS
(CASE 41 1500 cc IV)

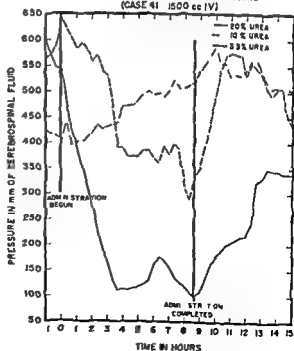


Fig 3 Comparison of different concentrations of urea solution given over an 8 hr period by slow intravenous drip (Case 41). The 3.3 per cent solution caused a slight rise in pressure whereas the 10 per cent and the 20 per cent solutions produced a fall. Excessive dehydration was caused by the 20 per cent solution.



Fig 2 Comparison of the effect of intravenous administration of identical volumes (256 cc) of 50 per cent sucrose and 30 per cent urea (Case 25). In the 2 rows of photographs the condition before injection of the hypertonic agent is illustrated on the left. The middle photograph in the upper row represents the maximum reduction following sucrose which occurred 30 min after injection. The effect of sucrose had disappeared almost completely within 1 hr as is shown by the third photograph in the upper row. The result of injecting urea as seen after an interval of 2 hrs is shown by the lower right photograph. The effect was near maximal at this time. The crater like area persisted for another hour and the return to the preinjection condition in which the mass was bulging required the lapse of another 2 hrs.

of 1500 mg/kg though it is well tolerated is greater than would be needed for clinical purposes. Very satisfactory results have been achieved with doses ranging between 500 to 1000 mg/kg.

Figure 2 demonstrates the hypotensive effect of urea in another manner and also provides a comparison of the results of the use of 30 per cent urea as opposed to 50 per cent sucrose. The three photographs in the upper part of the figure show the patient (Case 25) before and after the administration of 256 cc of 50 per cent sucrose. Radical resection of a recurrent right temporal oligodendroglioma had taken place 3 mo prior to the study.

Pseudomeningocele had developed at the site of subtemporal decompression. As may be seen in the center one of the upper 3 photographs (taken at the time when the sucrose effect was maximal) the sugar injection did not produce a significant reduction in the volume of the mass. (On another occasion the same volume of 50 per cent dextrose produced an even lesser effect). The 2 photographs in the lower part of the figure illustrate the effect of an identical volume (256 cc to 1000 mg/kg) of 30 per cent urea intravenously. The left photo was taken before injection; the right one shows how the bulge was converted into a crater like depression by the end of 2 hr. The crater persisted for another hour and there was still some noticeable effect of the urea injection at 11 hr. On a subsequent occasion it was found necessary in order to reproduce the decompressive effect of the above specified amount of urea to allow 87 cc of cerebrospinal

fluid to escape from the lumbar puncture needle. It should be pointed out that the comparison of sucrose and urea was made on an equal volume rather than on equimolar basis. If one had wished to give an amount of 50 per cent sucrose which contained the same number of molecules as 256 cc of 30 per cent urea it would have been necessary to inject the obviously excessive and dangerous amount of 876 cc.

The evidence which has been collected thus demonstrates conclusively that cerebrospinal fluid pressure may be lowered effectively with the aid of urea. Single doses which are sufficiently large to bring initially elevated pressures down to negative levels are still within the range of safety and are well tolerated. The secondary rise in pressure which has been reported as occurring following administration of 50 per cent dextrose has not been a problem with urea.

Having satisfied ourselves as to the efficacy of urea as an agent for reducing intracranial tension we turned more recently to the problem of determining suitable schedules for prolonged administration. Our studies have shown that the drop in cerebrospinal fluid pressure is proportional to urine output and urea nitrogen excretion. This indicates that diuresis is the primary mechanism of action in producing the hypotensive effect. Water loss and dehydration cannot be maintained indefinitely so the problem resolves itself into one of determining what degree of dehydration spread over what period of time will be of greatest net benefit to the patient in terms of reduction of intracranial pressure. We know from experience that repeated doses of urea administered several times daily over a period of weeks, continue to be effective. However, since the effect lasts for a matter of 3 to 6 hr with moderate doses such a regimen would merely produce intermittent periods of pressure reduction. Is there any way in

UREA FOR THE REDUCTION OF INTRACRANIAL PRESSURE
- EFFECT OF DIFFERENT CONCENTRATIONS
(CASE 41, 1500 cc IV)

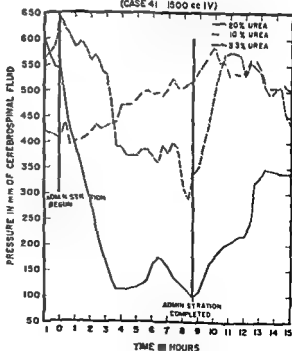


Fig 3 Comparison of different concentrations of urea solution given over an 8 hr period by slow intravenous drip (Case 41). The 3.3 per cent solution caused a slight rise in pressure whereas the 10 per cent and the 20 per cent solutions produced a fall. Excessive dehydration was caused by the 20 per cent solution.

which more sustained effect could be obtained without serious difficulties due to dehydration?

Figure 3 illustrates one attack on this problem. On 3 successive occasions the same patient (Case 11) received 1500 cc of urea solution by intravenous drip during an 8 hr period. Different concentrations of urea were used on the different occasions, namely, 3.3 per cent, 10.0 per cent and 20.0 per cent. Surgical removal of left fronto-temporal glioblastoma had been carried out 6 mo prior to the time of these studies and the patient had been comatose for about 1 mo. The curves indicate that the 10 per cent and 20 per cent solutions produced marked pressure reduction whereas the 3.3 per cent solution actually appeared to cause a slight increase in pressure. The 15 hr urinary output following the 3.3 per cent, 10.0 per cent and 20.0 per cent solutions were 1700 cc, 3000 cc and 6700 cc respectively. It should be emphasized that the 1500 cc dose of 20 per cent urea (1400 mg/kg) which was administered over an 8 hr period is definitely not a recommended dose. It produced a very marked reduction in pressure and maintained it well (approximately 50 per cent of the initial pressure) but it caused a marked state of dehydration which it was necessary to combat with vigorous measures at the conclusion of the study.

The data in Figure 3 suggest that an optimal concentration of urea for prolonged administration may be somewhere between 3.3 per cent and 10 per cent. An ideal concentration probably would be one which though administered in large volumes would nevertheless produce a small net fluid loss. Such a concentration might serve to maintain a state of decreased pressure with minimal adverse effects from dehydration. The search for such a concentration is continuing.

Therapeutic application of urea was not the primary purpose in the early phases of this investigation, since it was important first to test the efficacy and safety of the drug. However, after it had been demonstrated that urea could be administered in large amounts without untoward effect we began employing it routinely in cases of acute postoperative cerebral edema and in those preoperative cases when temporary control of intracranial pressure was essential. Dramatic improvement when urea was life saving occurred on several such occasions.

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ELECTRICAL STIMULATION OF THE HUMAN CEREBELLUM*

NICHOLAS WITZEL AND R. S. SNIDER

Stimulation of the cerebellum in the human has not been reported frequently. We have been able to stimulate the intact human cerebellum in 9 cases. Our results differ from those obtained by Pool in the only previous report but the circumstances of stimulation and types of stimulation are not comparable.

The present work arose from earlier basic studies on the cerebellum. As is well known, there are certain so-called sensory areas such as tactile, auditory, and visual which exist in the cerebellum and which are related to similar functional areas of the cerebrum. Electrical stimulation of these areas in animals may cause alterations in the EEG in certain cerebral areas. Changes produced by such stimulation may cause a reduction of frequency, i.e. slowing or under a differing set of experimental conditions or stimuli, low voltage fast activity may be initiated. Electrical stimulation of the cerebellum has also been shown to stop seizure activity initiated by electrical stimulation of the cortex. There is an adequate anatomical foundation for cerebellar projections to the motor and sensory areas of the cerebrum as well as to the reticular areas of the brain stem. Because of this background, we have felt that an experimental approach to these problems in the human was justified. Accordingly, we have whenever practicable stimulated the exposed cerebellum and have recorded changes in the electroencephalogram through conventional scalp electrodes. The patients studied have had various portions of the cerebellum exposed in routine neurosurgical procedures and have been free from signs and symptoms of cerebellar disease. Some of the procedures have been done entirely with a local anesthetic of 1 per cent xylocaine but most have been operated upon with the local anesthetic plus nitrous oxide oxygen or trilethene through an endotracheal tube for the convenience and comfort of all concerned. Anesthesia has been kept at such a level that there are minimal EEG alterations.

A Grass Model III C electroencephalograph was used with scalp electrodes placed on the available scalp. Stimuli were applied to the pia overlying the available cerebellar cortex using a bipolar electrode with electrical stimuli being generated by a Grass model 3A stimulator at parameters varying between 5 and 25 volts and frequencies between 1 and 300/sec. Points which caused changes in the EEG were suitably marked and photographs then taken. Observation of any motor movements were also made as were changes in blood pressure, pulse and respiratory rates.

In one instance we were able to expose most of the anterior lobe. In most of the others the exposure was such that the tonsillar portion of the posterior lobule and the regions immediately adjacent to them and/or the flocculonodular area were available.

The EEG effects which were observed usually lasted for several seconds following withdrawal of the stimuli and were free of any accompanying motor movement. Single shock stimuli were ineffective and it was necessary to irradiate the cerebellar cortex for from 1 to 5 sec. It was found that elec-

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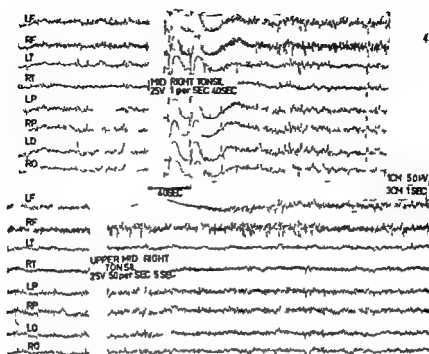


Fig 1 Electrical stimulation of pial surface of right tonsil of cerebellum Voltage duration and frequency are given Scalp recording from following sites LF & RF left and right frontal LT & RT left and right temporal LI & RP left and right parietal LO & RO left and right occipital Note enhancement of LF & RF voltage as a result of tonsillar stimulation

trical stimulation of the tonsillar area could enhance the amplitude of the fast activity recorded from the cerebral hemispheres In 2 cases this enhancement was limited to the frontal regions of the cerebral cortex Although enhancement of fast activity could be observed following anterior lobe

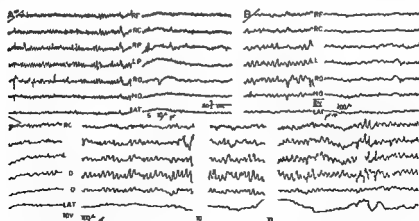


Fig 2 Electrical stimulation of pial surface of anterior lobe In (A) note that there is a reduction of slow waves (B) same as (A) While in (C) there is increased slowing Note removal of 10 sec strips from continuous tracing which can have fast activity super imposed on it Scalp leads over cerebral hemispheres were as follows RF RC RP and RO right frontal central parietal and occipital areas LP LAT and MO left parietal anterior temporal and motor (precentral) areas

stimulation the usual situation was the changing of fast activity to higher voltage slow waves especially in the range of 1 to 5 sec

In no case were we able to induce so called seizure activity in the cerebral cortex as a result of cerebellar stimulation Figures 1 and 2 are examples of the 2 kinds of LEC alterations which were observed

Although some variation in the results occurred in the various cases it is our impression that the following statements are justified

1 Electrical stimulation of the anterior lobe induced reduction of voltage and slowing of the cerebral LEC patterns

2 Stimulation of the tonsillar portion of the paramedian lobule produced increased voltage and frequency of EEG patterns

3 Single shock stimuli were ineffective but fractionation often produced effects which lasted many seconds in the poststimulatory period

4 It was not possible to produce cerebral seizure activity by cerebellar stimulation

5 The above effects were elicited without any visible signs of motor response

CEREBRAL METABOLIC STUDIES OF HYPOTHERMIA IN THE HUMAN*

JOHN E. ADAMS HENRY ELLIOT VIOLETTE C. SUTHERLAND EDWIN J. WYLIE AND ROBERT D. DUNBAR

Although hypothermia is being used with increasing frequency in cardiac vascular and neurological surgery many of the basic physiological changes occurring in the hypothermic state are as yet incompletely understood The nature of and tolerance for the profound acidosis resulting from hypothermia for example and the alteration in cerebral metabolic processes in relationship to the utilization of oxygen and glucose have not yet been adequately determined That cerebral tissues have a lowered oxygen requirement in the hypothermic state is now well established on the basis of both *in vitro* and *in vivo* studies Whether this decreased demand for oxygen is related merely to a lower cerebral metabolic rate or whether there is an actual alteration in the oxidative processes is unknown The majority of previous investigations concerning cerebral metabolic changes in hypothermia have been done in animals and scant human data are available The present study was undertaken because of the foregoing considerations

The patients studied were either undergoing neurosurgical procedures or were being operated upon for aortic aneurysms They were anesthetized with ether which was the anesthetic agent used throughout the surgical

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procedure unless the degree of hypothermia made additional anesthesia unnecessary. Preliminary premedication consisted of demerol and atropine. When the anesthetic level anticipated as necessary for the surgical procedure was attained a control cerebral blood flow study was done. It soon became apparent that it was necessary to administer the nitrous oxide mixture for 15 min to obtain equilibration between the brain and the cerebral venous blood in the hypothermic state. The control blood flow study was therefore also extended to 15 min instead of terminating it after the usual 10 min. The patients were then cooled in a refrigeration blanket the arteriolar needle being kept in place to provide continuous measurement of mean arterial blood pressure. When the desired temperature was attained a second cerebral blood flow determination was carried out. A closed gas exchange system was utilized so that an equivalent amount of ether was administered both during the control and the experimental blood flow study. It was found that a mixture of 15 per cent nitrous oxide and 85 per cent oxygen yielded better uptake curves than did the mixture usually used for cerebral blood flow determinations.

Blood oxygen and carbon dioxide analyses were made in duplicate by the manometric technique and Van Slyke and Neill¹ as modified by Kety and Schmidt.² These analyses were made at ordinary room temperature. It is probable that for greater accuracy the analyses should be made at temperatures corresponding to the temperature of the blood at the time the sample is obtained. We also assumed possibly incorrectly that the partition coefficient remains at unity at low temperature as Kety³ has shown it to be at normal temperatures. Glucose was determined by the iodimetric titration method of Somogyi.⁴ Clutamic acid and glutamine were measured by the microbiologic method of Harper⁵ and lactic acid by a modification of the method by Miller and Muntz.⁶

The values for cerebral blood flow and the cerebral metabolic rates for oxygen, carbon dioxide, etc., are expressed in cc/100 gm of brain/min. The values for glucose, lactic acid, glutamic acid and glutamine are expressed in mg/100 gm of brain/min.

The results for cerebral blood flow and metabolic rates are tabulated in Table 1. A positive sign represents uptake by the brain, a negative sign represents release by the brain. Hypothermia caused a reduction in cerebral blood flow in all but 2 patients (H. K. & H. C.). In these 2 the mean arterial blood pressure was higher at the lowered temperature whereas a significant drop occurred in all others. Consequently it seems probable that the systemic blood pressure is the most important single factor controlling blood flow at hypothermic temperatures. A significant increase in cerebral vascular resistance associated with hypothermia occurred. Whether this was due to decrease in caliber of cerebral vessels or to increased viscosity of blood can not be stated. The cerebral metabolic rate for oxygen was lowered. The high CMRO₂ shown for patient M. R. in the control period may be attributable to the patient's age since it is probable that prepubertal children have higher cerebral metabolic rates than adults. It is apparent that utilization of glucose decreases at hypothermic temperatures although the values given are not statistically significant. The expected release of lactic acid by the brain in the control period occurred in all but 2 patients. This release was less at hypothermic levels.

Table 1

TREATMENT	CONTROL						EXPERIMENTAL									
	CM	CAIRO	CAIRO	CAIRO	CAIRO	CAIRO	CM	CAIRO ₂	CAIRO	CAIRO	CAIRO	CAIRO	CAIRO	CAIRO	CAIRO	CAIRO
ACT	68	+49	-17	112	0.6,	290	26.2	+14	-0.75	3.20	0.12	3.12	0.19	-3.16	-2.51	0
AC	16	+32	-30	12	0.93	+115	-0.9	12	+0.9	290	27.2	+107	-0.18	3.12	0.19	-3.16
RM	185	+23	-19	12	0.88	+16	-3.1	300	30.8	+12	-0.73	2.10	0.60	-2.52	-0.9	0
IB	196	+28	-24	166	0.87	+39	-2.8	+0.1	+1.1	295	130	+22	-1.82	1.93	0.81	-2.62
J	559	+25	-3	12	1.1	+67	+1.9	+0.21	+0.18	290	300	+0.1	-0.19	2.53	1.2	-0.58
HA	1751	+13	-17	196	1.92	+681	-16	-0.37	+0.73	295	515	+12	-1.0,	2.11	1,	+3.31
RC	602	+15	-22	149	1.16	+16	-0.1	+0.12	+0.61	295	391	+0.7	-0.19	2.07	0.61	+7.21
HC	116	+23	-23	163	1.0	+178	+18			31	580	+21	-1.91	1.03	1.1	+1.9
MAN	115	+260	-271	143	1.10	+193	-1.05	+0.31	+0.71		183	127	-1.09	2.35	0.9,	+1.13
Standard dev of MAN	±6.09	±1.01	±0.91	±0.24±0.7	±1.6	±2.08	±0.16	±0.13		±1.1	±0.68	±0.76	±0.75±0,	±2.5	±1.6	±0.02

Table 2 Arterio Venous Differences

	O ₂	CO ₂	IAC				ICU				IAC			
			CUU COSI	TIC ACID	TAMIC ACID	CUUA MINI	CUU COSI	TIC ACID	TAMIC ACID	CUUA MINI	CUU COSI	TIC ACID	TAMIC ACID	CUUA MINI
Mean	+1.40	-3.99	+7.33	-2.1	-0.11	-0.61	+3.37	-2.75	+2.63	-0.98	-0.03	-0.71	-0.03	-0.71
S	±0.3	±0.2	±1.3	±1.1	±0.14	±0.32	±0.1	±0.08	±0.9	±0.7	±0.008	±0.21	±0.008	±0.21

Table 3 Absolute Blood Levels

	CONTROL		EXPERIMENTAL		10 NORMALS	
	Λ	Λ	Λ	Λ	Λ	Λ
O ₂ Vol %	18.6	11.71	20.91	16.8	16	11.3
CO ₂ Vol %	18.79	2.36	11.96	17.22	48.5	53.9
Glucose M _h %	114.8	138.7	102.9	187.2	102.3	93.9
Lactic Acid M _h %	11.3	13.1	19.3	20.2	6.1	7.4
But Acid M _h %	0.70	0.66	0.26	0.29	0.1	1.5
Glutamic M _h %	8.59	8.12	6.12	6.81	9.2	9.6

In several patients cerebral blood flow could not be calculated due to inadequate uptake curves for nitrous oxide. Arteriovenous differences are therefore shown for the total group of 12 patients in Table 2. In general the A-V differences correspond with the cerebral metabolic rates in Table 1 and statistically are more convincing.

The mean values for the absolute levels of the various moieties studied are listed in Table 3. It is uncertain whether the higher values for oxygen indicate increased solubility of gases at lower temperature or whether they represent a metabolic alteration. Insofar as glucose is concerned it has been shown that plasma glucose levels are increased by hypothermia. The striking increase in blood levels for lactic acid under hypothermia is of great interest. We do not believe that this increase was caused by shivering since in most instances shivering was well controlled by the anesthetic agent.

DISCUSSION

These preliminary results in humans corroborate the findings of others that at hypothermic levels there is a decrease in cerebral blood flow and in the cerebral utilization of oxygen and glucose. Alterations in the arteriovenous oxygen levels in general were related to the rate of cerebral blood flow, but a decrease did occur with falling temperatures. Our findings are therefore in agreement with those of Bering *et al.*⁷ and at variance with the results obtained by Rosomoff⁸ who reported that the arteriovenous oxygen levels remain constant at reduced temperatures. In instances where the mean systemic arterial blood pressure either did not fall or rose at the level of lowered body temperature maintained in the experimental study the cerebral blood flow did not drop and similarly there was little change in the cerebral metabolic rates for oxygen.

The fact that hypothermia produces high blood levels for lactic acid is of interest and the source of this lactic acid is yet to be determined. We have carried out a few determinations of blood pH and have found as others have that the pH drops to remarkably low levels (7.0 to 7.2). It has

been suggested that this metabolic acidosis is caused by an increased $p\text{CO}_2$. Although our data are incomplete they suggest that the acidosis may be related in part to the high blood level of lactic acid since at the measured level of pH lactic acid, not lactate is present.

The fact that there is a definite decrease in the liberation of lactic acid from the brain in the hypothermic state and in some instances a utilization of lactic acid raises the possibility that anaerobic oxidative processes may be operative. Our findings in regard to lactic acid levels are in variance with those of Loughheed and Kahn⁹ who observed no change in lactic acid levels as long as their animals were not anoxic. We feel certain that our patients were not anoxic not only on the basis of their appearance but also because of the blood oxygen and CO_2 levels.

Further studies are in progress to elucidate some of the problems raised by these findings.

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INTRACRANIAL IMPLANTATION OF LIQUID PLASTIC IN THE EXPERIMENTAL ANIMAL (MACAQUE) POSSIBLE CLINICAL APPLICATION IN SURGICAL MANAGEMENT OF INTRACRANIAL ANEURYSMS*

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The purpose of this work is the search for a relatively innocuous liquid or semi liquid agent which might be used intracranially in reinforcing saccular aneurysms without occlusion of the parent vessel and with minimal cerebral scarring effect.

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MATERIALS

Pure methyl methacrylate or polyethylene, the plastic chosen for this study, was dissolved in 1 of several organic solvents and implanted in 11 experimental animals (Miquie). These agents included propyl acetate, ethyl acetate, trichloroethylene, acetone, acetone with diethyl phosphite and chloroform.

Implantation in the temporal area was carried out in each animal. In some the liquefied plastic was placed in the subdural space about the optic nerve and olfactory tract or in the Sylvian fissure. In others a cerebral defect was created in the inferior frontal and anterior superior temporal lobes measuring 1.5 cm, sparing the middle cerebral complex which was deliberately left to bridge this space. These vessels were then embedded by filling the cerebral defect with liquid plastic.

RESULTS

Methyl Methacrylate and Acetone 1) No. 1108 This combination was instilled about the right optic nerve along the frontal base and in the Sylvian fissure. On sacrifice 5½ wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vasculature without evidence of thrombosis of major vessels. The plastic presented a yellowish white appearance and was flint like in consistency. It was loosely attached by filmy adhesions to meninges of softening extending 0.5 cm in all directions.

2) No. 1595 Methyl methacrylate and acetone was instilled into a cerebral defect on the right side measuring 1.5 cm in diameter about the middle cerebral vessels which were deliberately left to bridge this space. On sacrifice 5 wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed no evidence of thrombosis of the major vessels traversing the cuff of plastic placed about them in the Sylvian region. The plastic was densely involved in scar tissue, presented a cratered appearance and was flint like in consistency.

These two animals survived without evidence of neurological disability. The combination of methyl methacrylate and acetone while difficult to handle in the liquid state, congealed quickly the surface first the interior of the mass remaining liquefied. This characteristic permitted molding of the compound into a spherical shape which could then be cut with scissors and placed as a cuff about the middle cerebral vessels.

Methyl Methacrylate and Acetone with Diethyl Phosphite 1) No. 1158 This combination was instilled about the right optic nerve and olfactory tract and in the Sylvian fissure in the subdural space. On sacrifice 8 mo later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vasculature without evidence of thrombosis of major vessels. The plastic presented a yellowish white appearance and was flint like in consistency. Filmy adhesions bound it to the pia arachnoid.

2) No. 1216 Methyl methacrylate and acetone with diethyl phosphite was instilled into a cerebral defect on the right side measuring 2 cm in diameter, the middle cerebral vessels bridging this space and consequently completely embedded in the plastic. This animal was sacrificed 6 wks later.

following which India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels embedded in the plastic. The plastic yellowish white in appearance and flint like in consistency was densely involved in scar the fibrosis response considerably greater in degree than that seen with methyl methacrylate and acetone. These 2 animals presented no neurological abnormalities during the survival period.

The physical characteristics of methyl methacrylate and acetone with diethyl phosphite vary in only 1 major respect from methyl methacrylate with acetone. The former appears to be more liquid and from a technical standpoint is more easily handled. However it hardens as quickly as methyl methacrylate and acetone.

Methyl Methacrylate and Tri Chloroethylene 1) No 4806 This combination was instilled about the right optic nerve the olfactory tract and in the Sylvian area in the subdural space. On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out. Subsequent examination of the brain disclosed no evidence of thrombosis of major vessels. However extensive softening of the frontal and temporal lobes was apparent. The plastic conforming to its environment had precipitated a dense adhesive process and presented a yellowish white cratered appearance.

2) No 4703 Methyl methacrylate and tri chloroethylene was instilled into a 1.5 cm defect involving the inferior frontal and anterior temporal lobes on the right side the middle cerebral vessels left to bridge the space and consequently completely incorporated in the plastic. On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels. The plastic was densely incorporated in scar tissue the fibrosis response considerably greater than with either of the 2 preceding plastic solvent combinations. Additionally softening of the adjacent brain substance extended approximately 1 cm in all directions.

These 2 animals presented a stormy postoperative course generating large quantities of fluid beneath the scalp at the site of operation. In each instance right pupillary dilatation and moderate paresis of the left extremities was present though improving at the end of the survival periods. The combination of methyl methacrylate and tri chloroethylene is easily handled technically since it remains liquified for an extended period of time. In each of the above instances the material had not solidified as of approximately 30 min after instillation.

Methyl Methacrylate and Propyl Acetate 1) No 4679 This combination was instilled about the right optic nerve olfactory tract and in the Sylvian area subdurally. On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the cerebral vascular tree without evidence of thrombosis of major vessels. The plastic which presented a yellowish appearance was densely bound to the adjacent structures by scar tissue. Additionally softening in the inferior frontal and anterior temporal area was apparent.

2) No 4756 Methyl methacrylate and propyl acetate was instilled into a 1.5 cm defect involving the inferior frontal and anterior temporal region encompassing the middle cerebral vessels left to bridge this space. On sacrifice 11 wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the cerebral vascular tree showed no evidence of thrombosis of major vessels. The plastic densely involved in scar tissue and surrounded by an extensive area of softening presented a yellowish appearance with cratered and irregular, and flint like in consistency.

These 2 animals were quite ill for several days and weakness of moderate degree of the left extremities was noted. Considerable quantities of fluid generated beneath the scalp at the operative site. At the end of their survival periods almost full recovery from paresis of the left extremities had occurred.

The combination of methyl methacrylate and propyl acetate because of a prolonged liquid phase is easily manageable from a technical standpoint. However as of 15 min after instillation in either of the above instances no solidification had occurred and the wounds in each instance were closed without waiting for congealing.

Methyl Methacrylate and Ethyl Acetate No 4640 This combination was instilled about the optic nerve olfactory tract and in the Sylvian area subdurally on the right side. On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels. The plastic presenting a yellowish white appearance and flint like consistency was densely bound to the adjacent structures by adhesions and softening of moderate degree involved the inferior frontal and anterior temporal lobes.

This animal's postoperative course was also somewhat stormy. Right pupillary dilatation and paresis of the left extremities of mild to moderate degree was evident. However at the end of its survival period it had completely recovered. The combination of methyl methacrylate and ethyl acetate congeals slowly and as of approximately 15 min after instillation had not hardened.

Methyl Methacrylate, Acetone and Tincture Merthiolate No 4507 This combination was instilled into a 1.5 cm cerebral defect involving the inferior frontal and anterior temporal area on the right side about the middle cerebral vessels left bridging this space. This animal partially recovered from anesthesia but then regressed and died 21 hr later. Postmortem examination after India ink irrigation of the cerebral vascular tree through the right common carotid artery disclosed no evidence of thrombosis of major vessels. However approximately 10 cc of subdural fluid of anthrochromic character was encountered. It was felt that this accounted for the animal's demise. The plastic had completely solidified presented a pink appearance and its surface was smooth unlike the cratered irregular appearance of specimens left in the animal for several weeks.

Methyl Methacrylate and Chloroform No 4512 This combination was instilled in the left inguinal area about the femoral artery vein and nerve. Additionally a small amount was instilled into the Sylvian area subdurally on the left side. Methyl methacrylate and acetone was instilled subdurally

in the Sylvian area on the right side and also about the right femoral artery, vein and nerve in the inguinal region. During the first 4 days following surgery the animal remained lethargic and inactive though gradually improving. On the fifth day the inguinal wounds interrupted bilaterally, and on the eighth day, the animal generally improving but with considerable drainage from its inguinal wounds was returned to the operating room. The left femoral artery and vein were found grossly thrombosed and marked tissue reaction surrounded the plastic block. On the right side (acetone) the femoral artery and vein were intact. Only minimal tissue reaction had occurred although the wound was obviously infected. One week later the animal died apparently from intracranial complications and was discarded without our knowledge.

SUMMARY AND CONCLUSIONS

Methyl methacrylate dissolved in 1 of several organic solvents was implanted intracranially in a series of 11 monkeys. Death occurred in 2 (chloroform and acetone with tincture of methylolate). No neurological sequelae were observed in the 2 animals in which methyl methacrylate and acetone, or in the 2 animals in which methyl methacrylate and acetone with dicetyl phosphate had been implanted. The remaining 5 exhibited either transient neurological deficits or sequelae which were reduced to a minimal character by the time of sacrifice.

Least local reaction occurred in the case of the acetone combination. A slightly greater response resulted with acetone and dicetyl phosphate. Marked sclerosis occurred with the remainder of the solvents used. The presumption is made that intracranial implantation of chloroform compound would produce a severe reaction based on observations of femoral artery and vein occlusion and subjacent sclerosis of extreme degree. Though no interruption of the pia arachnoid barrier occurred in subdural implantation softening of variable degree involved the adjacent cerebral tissue. Least reaction occurred with acetone. A much greater degree of softening occurred with the other solvents. In no instance in the 9 surviving animals was there evidence of thrombotic occlusion of major vessels lying either adjacent to or traversing masses of plastic. Patency of the cerebral vascular tree was best demonstrated by postmortem India ink injections.

From a technical standpoint the acetone combination was most difficult to handle because of stickiness and jelly like consistency. The remaining solvents produced a more easily manageable mass because of a more liquid character. However they congeal slowly because of reduced volatility of the organic solvent. It is believed that this reduced volatility prolonged the irritating effect of the organic solvent accounting for the greater degree of reaction observed on cerebral tissue especially when the pia arachnoid barrier is interrupted. With the acetone combinations high volatility of the solvent permits rapid hardening of the plastic and consequently a much shorter duration of contact of the solvent with the cerebral tissue.

It is recognized that experience with these solvents is limited. However these preliminary results suggest acetone or perhaps acetone with dicetyl phosphate for increased scarring effect as the least irritating of the organic solvents. Furthermore since thrombosis of cerebral vessels lying adjacent to or traversing masses of plastic does not appear to occur it is considered

surgically feasible to encompass intracranial vascular aneurysms as a reinforcing measure where sacrifice of the parent vessel might be complicated by severe neurological disability or in those instances where ligation of the aneurysm is followed in a high percentage of cases by thrombotic occlusion of the parent vessel

SUPRAPINAL RECESS*

ORLANDO J. ANDY AND JAMES S. BROWN

Deformities in the configuration of the posterior limits of the third ventricle, secondary to both infra and supratentorial tumors, has been given a great deal of attention. However very little study has been given to its configuration in patients without tumors thus leading to the occasional difficulty in recognizing the pathologic patterns.

The posterior wall of the third ventricle beginning inferiorly, consists of the arculus of the aqueduct of Sylvius, posterior commissure, pineal recess, habenular commissure and the suprapineal recess. The suprapineal recess consists of an evagination or diverticulum of the ventricular epithelium and usually contributes to a major portion of the posterior wall. A membranous pouch should lend itself very well to displacements and deformities secondary to nearby lesions and thus be of diagnostic value. However, under normal conditions membranous diverticula present a variety of patterns and thus in this location may detract from its diagnostic value. In this study, emphasis is placed on characteristics of the suprapineal recess under conditions not complicated by tumors.

Factors being evaluated are (1) length and (2) height of the recess (3) angle between the recess and the aqueduct and (4) distance between the recess and aqueduct.

METHOD

This study is based on 31 pneumoencephalograms. Patients varied in sex, race and age (3 to 64, Fig. 2). They were selected from the neurological and neurosurgical services of a Veterans and a University Hospital. Under local anesthesia pneumoencephalograms were done in the sitting position with the neck inflexed approximately 45 degrees. A lateral x-ray was taken at a tube plate distance of 10' after injecting 10 cc. of air.

Outline drawings were made of the aqueduct and the posterior wall of the 3rd ventricle (Fig. 1) and from these 4 measurements (Fig. 2) relative to the suprapineal recess were determined as follows:

(D — D) Height of suprapineal recess a measurement between the upper and lower lips of its mouth. This mouth is arbitrarily determined by a line running through the habenular commissure perpendicular to Reid's base line.

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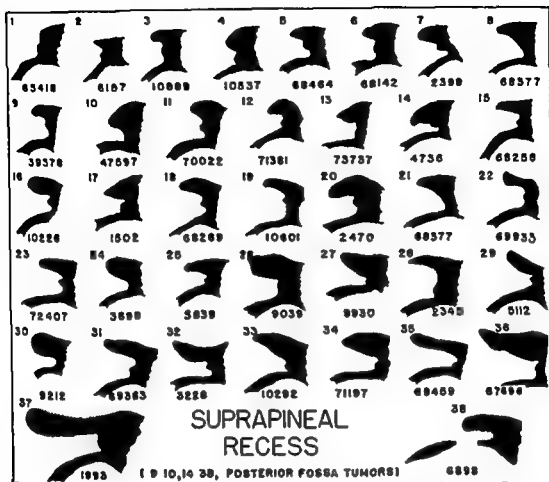


Fig 1 Tracings of the posterior wall of the 3rd ventricle and rostral aqueduct. The first 31 cases are in order of increased length of suprapineal recess. Cases 14 and 38 represent pontine gliomas. Cases 9 and 10 represent a mid line cerebellar astrocytoma and a 4th ventricular cyst respectively.

(C — — C) Length of the suprapineal recess: the distance from a point mid way between (D — — D) to the most distal portion of the diverticulum.

(A) Suprapineal recess aqueduct angle is determined by tangents to the most ventral surface of the recess and the most dorsal surface of the aqueduct with the pineal recess as its point of origin.

(B — — B) Suprapineal recess aqueduct distance: measurement between the 2 points of tangency as determined in (A).

RESULTS

Considerable variation in the size, shape and position of the suprapineal recess was found in patients without space-occupying intracranial lesions. These variations appeared to be independent of the age, sex and race of the patient.

The shape varied from a blunt or very shallow evagination (Fig 1 1) to an elongated sac (Fig 1 37). The elongated recesses either curve upward around the splenium of the corpus callosum downward or remain straight. Its configuration varied from a narrow to a very wide sac (Fig 1). Length

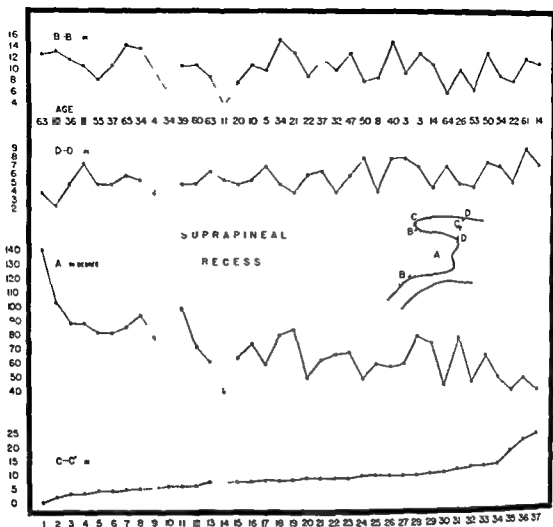


Fig 2 Three measurements in mm and 1 in degree as illustrated in the insert are plotted from the air study of 37 patients. Cases 9, 10, and 14 have posterior fossa lesions; the rest are normal. They are arranged in sequence of increased suprapineal recess length (C — C) length of recess (A) angle between recess and aqueduct (D — D) height of recess (B — B) recess aqueduct distance.

(Fig 2 C — C) of the recess ranged from 1 to 24 mm. Height (Fig 2 D — D) varied from 2.5 to 9 mm. The recess aqueduct distance (Fig 2 B — B) varied from 6 to 16 mm. The angle (Fig 2 A) as previously described between the recess and the aqueduct varied from 40 to 140°. As the length of the recess increases there is a concomitant decrease in the angle (Fig 2). There is also a suggestive concomitant decrease in the recess aqueduct distance (B — B) however it is not as marked as the angle alteration. The height of the pineal recess is the most stable measurement which undergoes very little change with changes in the pouch's length. However there may be a tendency to increased height with increased length. In order to demonstrate deviations from the 31 normals, 4 pathological cases have been added (9, 10, 14, and 38, Figs 1 and 2). Cases 14 and 38 are pontine gliomas which are considered in their respective positions in the chart (Fig 2) according to recess length show marked decrease in angle size and recess aqueduct distance in comparison to the normal range. Case 38 according to recess length should be in the same position as 31 and

would show an angle of 20 degrees thus being completely out of line with the apparent normal trend of the angle curve Case 10 is a cyst of the 4th ventricle with obvious deviations from the general trend of the curves as shown in Fig. 2 Case 9 is a child with an astrocytoma of the 4th ventricle which is presented as an exception since it did not produce detectable changes by the criteria presented above However 1 yr later the child returned and was found to have very extensive changes in this region (Courtesy of Dr Charles Neill)

DISCUSSION

Others¹⁻³ have attempted to use the suprapineal recess as a measurement reference point in the diagnosis and localization of both infra and supratentorial lesions Wilson and Lutz² have drawn a line along the base of the anterior fossa to the anterior clinoid and another from the latter to the suprapineal recess This angle was normally 140 degrees Epstein¹ made measurements from the suprapineal recess to the anterior inferior angle of the 3rd ventricle This was found to be 4.2 to 1.7 cm Measurements were also made from the recess to the midline which ranged from 0.5 to 0.6 cm Furthermore it is stated that no changes were found in the latter measurements in cases of posterior fossa tumors He cites Ecker's³ article as lending support to his thesis

To the present authors it seems that measurements as suggested by Epstein and Wilson and Lutz presuppose that the suprapineal recess and structures immediately surrounding it in the brain stem act as a unit and move as a mass when displaced The anatomical differences such as consistency shape and contents of the structures under question make this supposition improbable Detailed measurements of the suprapineal recess with reference to its immediately surrounding structures reveals that these structures do not move en masse Application of the 4 criteria of measurement to the pathological ventricular patterns published by others^{3,4,6} support these observations

SUMMARY

The pneumoencephalographic size shape and position of the suprapineal recess was described and illustrated with reference to its immediately surrounding structures in 31 patients interpreted as having normal air studies The suprapineal recess in this group presented a great deal of variation However 4 measurements of this recess are presented in the form of a graph which can be employed to detect deviations from the expected normal

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It is known that the motor cortex receives fiber projections from the nucleus ventralis lateralis of the thalamus. The latter in turn derives its afferent supply from the red nucleus and more directly from the cerebellum. It is also believed that the globus pallidus also connects with the nucleus ventralis lateralis. Considering the multiple connections of the red nucleus with the tectum, zona incerta, subthalamic nucleus and substantia nigra, and of the globus pallidus with the striatum, one would assume that the nucleus ventralis lateralis would serve as an important relay station to the motor cortex and would exert considerable influence upon the activity of the cortical motor neurones. For instance, lesions in the cerebellum give rise to hypotonia, athetosis and synergic manifestations. Similarly, diseases of the basal nuclei are frequently accompanied by undesirable movements.

On the basis of clinical observations and the anatomical connections of these subcortical structures, it was postulated that certain neuronal pathways may be significant in relation to motor function.¹

This report consists of the results from a series of experiments in which the effect of the discharge from the nucleus ventralis lateralis upon the activity of the cortical motor neurones was investigated.

In all experiments electrical stimulation was applied to the nucleus ventralis lateralis and to the motor cortex of the cat. The recording electrode which was capable of detecting action potentials from single cortical cells was a glass pipette having an outside diameter of about 0.5 micron at the tip and filled with 3 M KCl solution. The microelectrode which was inserted into the motor cortex was then connected to the amplifier-oscilloscope recording unit. Recording of the slower electrical activity adjacent to the cortical cells under study was simultaneously carried out by means of a large electrode on the cortical surface.

When a microelectrode was inserted into the grey cortex it recorded spike action potentials of the single cortical neurones. The spikes recorded were variable in shape, presumably due to the location of the tip of the recording electrode in reference to the surface membrane of the cell.

Thus, if the tip was outside but very near to the cell membrane, spikes which were predominantly negative in electrical sign were recorded. If the tip was in direct contact with the cell membrane, it recorded positive-negative spikes. And if the tip was situated inside a cell, a monophasic positive spike was recorded.

When a cortical unit was discharging spontaneously, in prolonged times a single stimulus to nucleus ventralis lateralis would arrest the discharge of the unit for as long as 100 msec. after which the discharge of the unit recurred. The temporary arrest of spontaneous unit spike activity in response to synaptic volleys seemed to be a common occurrence since it was observed not only in the motor cortex but also in the visual and somatosensory cortex.

However, in the motor cortex when spike activity occurred only in response to stimulation, it was found that the spike response was also suppressed by a preceding stimulus to the nucleus ventralis lateralis. This is in

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contrast to the observations obtained in the somatosensory cortex where the neuronal responses were facilitated by preceding stimulation of an unspecific thalamic nucleus.¹

Further the slow potential response evoked by direct stimulation of the motor cortex was also suppressed by a preceding stimulus applied to this thalamic nucleus. Thus when stimulation of the motor cortex elicited a slow potential response and a unit spike discharge, a preceding thalamic volley would cause a reduction in amplitude of the slow potential and disappearance of the unit spike. The inhibitory effect would persist for 40-80 msec.

In conclusion it has been shown that the discharge of nucleus ventralis lateralis of the thalamus inhibits the activity of the cortical neurones in the motor cortex. Since the principal efferent pathway for voluntary movement has its origin in the motor cortex, it seems logical to suppose that the function of this thalamic nucleus is primarily inhibitory or regulatory in relation to motor activity.

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CERTAIN EFFECTS OF PERMANENT PITUITARY STALK INTERRUPTION IN THE HUMAN WITH MAMMARY CANCER*

GEORGE EHNI AND NYLEN E. ECKHART

It has been abundantly shown in several species that when the pars distalis is deprived of functional contact with the hypothalamus, it becomes dormant or at best operates at such a depressed level as to be incapable of sustaining certain of its endocrine dependencies. Ablation of pituitary function achieved by hypophysectomy has been shown to exert a favorable effect upon certain patients who have advanced metastatic mammary cancer. Since isolation of the human pituitary gland by permanent interruption of its stalk appeared likely to quench its function with less technical difficulty than attends gland removal and possibly with more certainty of functional ablation, it was decided to subject a small series of humans suffering from advanced metastatic mammary cancer to pituitary stalk interruption. This

*From the Neurological Surgery Service and the Section on Experimental Surgery of the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas. With the technical assistance of Jacqueline Claus in the hormone assays and Fern Garrett in the tests of thyroid function.

we accomplished by means of a right subfrontal approach followed by section of the pituitary stalk and placement of a roughly circular polyethylene stamp about 1.4 cm in diameter upon the diaphragma sella centered over the distal end of the cut stalk.

Of the 6 patients herein reported 5 had been previously treated with craniotomy and/or exogenous steroids and had either failed to show response or were in relapse following a period of improvement. In the remaining case pituitary stalk section was the initial treatment for the metastatic disease. This patient was the youngest of the group. She had an extremely short history (1 mo post mastectomy) and displayed widespread osteolytic metastasis with anemia. This latter patient was the only one menstruating at the time of stalk section.

Cortisone administration during and after surgery was given according to need rather than prearranged plan. Four patients received no cortisone before or during the operation but had it started in doses of 25 mg every 6 hr upon completion of the surgical procedure. Of these 4, 3 experienced serious hypotension during the first 12 hr following the operation and all responded to intravenously administered hydrocortisone. Two patients received 150 mg of cortisone intramuscularly prior to the operation and 50 mg every 4 to 6 hr thereafter. One of these patients developed significant hypotension within the first 12 hr following stalk section and required intravenous hydrocortisone.

All patients have required maintenance doses of 25 mg cortisone daily for optimum well being although for purposes of test it has been possible to withdraw cortisone from each while under observation in the hospital for periods of from 1 to 4 wks. Four of the 6 patients have required pitressin for the control of some degree of presumably permanent diabetes insipidus. No other endocrine products or dietary measures for control of electrolytes have been used to date though it is expected that additional replacement therapy may become necessary as end-organ failure (such as the thyroid) progresses.

The course of the neoplastic disease was gauged by serial roentgenograms, 24 hr urinary calcium excretions, serum calcium levels, serum alkaline phosphatase studies, hemoglobin determinations and by photographs and measurements of tumor masses. The effects upon thyroidal, adrenal and ovarian functions were also assayed.

RESULTS

Effects Upon the Neoplasm Table 1 displays the essential clinical data on the 6 patients comprising this report. It is to be noted that the longest period of observation is 7 mo so that no conclusion can be drawn as to whether benefits deriving from this operation compare favorably in duration with those produced by hypophysectomy, adrenalectomy or cortisone therapy. The clinical result in the first patient appears at this time however to compare favorably with the best responses produced in other instances of metastatic breast cancer by these other procedures. This patient is completely devoid of subjective complaint of any kind, has gained 24 lbs in weight, has developed no new skull or other lesions and the previously developed osteolytic areas throughout her skeleton have shown a marked blastic response. Prior to the operation her 24 hr urinary calcium excretions

Table 1 Clinical Data on Six Patients with Metastatic Mammary Carcinoma Submitted to Pituitary Stalk Section

PATIENT	METASTATIC SITE	CLINICAL STATUS	ENDOCRINE STATUS	SURVIVAL	PITUITARY STALK SECTION	
					CALCIUM EXCRETION	TUMOR CALCEA
1	Skeletal (lytic)	Bedridden bone pain vomiting, anemia	Intact Menstruating	7 months	Working pain free	No new lesions Strong blastic response in skeletal lesions Eucalcemia 24 hr urinary calcium fell from 510 mg to 17 mg at 24th week
2	Skeletal (lytic)	Barely ambulatory bone pain	2 years after menopause	5 months	Unchanged	Skeletal lesions advance Hypercalcaemia persists
3	Pleural skeletal (lytic)	Barely ambulatory bone pain	Surgical castrate	4 months	Working pain free	No new lesions Beginning blastic response in old ones 24 hr urinary calcium fell from 160 mg to 20-50-90 mg
4	Pleural nodal chest wall	Ambulatory painful swollen arm	Surgical castrate	3½ months	Bedridden near death	Progression in all lesions
5	Skeletal subdermal	Bone pain vomiting ambulatory	Surgical castrate	3 months	Working pain free	No new lesions Early blastic response in old ones Subcutaneous masses smaller 24 hr urinary calcium fell from 208 mg to 44 mg
6	Peritoneal hepatic nodal skeletal (blastic)	Barely ambulatory vomiting ascites	Surgical castrate	Dead 2¼ mo after operation		All lesions advanced

averaged 510 mg but since stalk section the value has consistently ranged between 30 and 50 mg. The third and fifth patients show calcium excretion reductions of the same order. They have developed no new skeletal or other lesions during the short period of postoperative observation and the blastic response noted roentgenographically in the already existing skeletal lesions though present is of a lower order in magnitude than in the first case. Three of the 6 patients, 1 of whom had skeletal metastases seemingly comparable in every way to those in patient Number 1, derived no benefit from the operation by any measurable criterion.

Effects Upon Target Organ Physiology Thyroid function has been evaluated by determination of the 24 hr uptake of orally administered I^{131} and by

determination of protein bound iodine. For only 2 of our cases do we believe that the period of observation has been long enough to have significance. Patient Number 1, in whom the best clinical result to date was obtained and who has been longest under study, shows no appreciable change in either the I^{131} uptake or the PBI. Contrasting with this patient is our Number 2 who was not clinically benefited by the operation but who showed at the end of 1 mo. in I^{131} uptake of only 1 per cent as contrasted with an initial level of 33 per cent. The diminution in her protein bound iodine was similarly striking. Observations in the remaining 4 patients suggest that thyroid function is decreasing in all but at a slower rate than reported by others for hypophysectomy.

The change in absolute eosinophil count was measured in response to ACTH (25 units given as intravenous infusion over 8 hr.) before and after stalk section. The drops were normal before surgery but 1 mo. after it there was little or no change in the number of circulating eosinophiles during the 8 hr. test. A moderate eosinopenia occurred when ACTH stimulation was repeated on the second day.

The 4 hr. glucose tolerance test done at monthly intervals after stalk section reveals extreme flattening of the pretreatment curve. This increased sensitivity to endogenous insulin is paralleled by a response to intravenously administered insulin (6 units) following which subconvulsive sugar levels resulted. Change in glucose tolerance as measured by these tests parallels that recorded by others for the hypophysectomized state.

Plasma corticosteroid levels and the 24 hr. urinary excretion of corticosteroids were determined pre and postoperatively after withdrawal for 48 hr. or more of the exogenous cortisone and determinations were repeated on successive days following administration of 25 units ACTH by 8 hr. intravenous infusion. The result in all cases was the same, that is, there occurred a drop from normal values of both urinary and plasma corticosteroids to almost immeasurably small levels following stalk section. This occurred within 3 wks. after surgery. During the 3 day ACTH stimulation test the increase in corticosteroid values of plasma and in 24 hr. urine collections on the first day was negligible. On the second day showed a slight increase in a few instances only, and on the third day in all cases approached normal values.

Patient Number 1 had normally functioning ovaries at the time of stalk section. Endometrial biopsy was done 3 and 6 mo. following operation. The material on both occasions showed glandular and stromal hyperplasia of the proliferative type. Vaginal bleeding has not appeared. This evidence of follicular stimulation without luteinization is not explainable by our material at the present time.

CONCLUSIONS

In a small series of patients with disseminated mammary cancer subjected to permanent pituitary stalk interruption the procedure provided amelioration of the disease of the same order of frequency and magnitude as hypophysectomy. During the 2 to 7 months of postoperative observation thyroidal depression in some degree has been noted in 5 of the 6 cases. All patients showed marked postoperative depression of adrenal function comparable to that reported for patients following hypophysectomy or pituitary necrosis. ACTH administration over several days evoked adrenocortical ac-

tivity in all subjects tested. Cortisone withdrawal while the patient was hospitalized for periods of 1 to 4 wks was tolerated but comfort and feeling of well being were adversely affected. In the 1 patient having normally functioning ovaries before stalk section cyclic bleeding has not reappeared but the endometrium shows estrogenic stimulation. This patient demonstrates the most dramatic regression of neoplasia and the concept of estrogen dependency determining breast cancer response is in her case at least unsupported.

These preliminary observations suggest that permanent pituitary stalk interruption merits more intensive study both as palliative treatment for metastatic breast cancer, and as a means of advancing understanding of the physiology of neoplasia.

AN EVALUATION OF HYPAQUE SODIUM (WIN 83083) FOR CEREBRAL ANGIOGRAPHY*

D. W. LINDNER, F. A. MARTIN, J. E. WEBSTER AND E. S. GURDJIAN

Since the acceptance of cerebral angiography as a relatively safe diagnostic procedure the merits of various media and the techniques of their administration have been reported. The purpose of this study was to evaluate Hypaque Sodium (WIN 83083) as a contrast medium comparing it with other media i.e. Diodrast, Thorotrast, Urokon and Renografin.

METHOD

Twenty two dogs were used in the study. Under nembutal anesthesia polyethylene catheters were placed in the femoral vein and carotid artery. Injections of the various contrast media were made via the carotid catheter, varying the amount, concentration and rate of injection. Then following the method of Broman and Olsson¹ for analysis of disturbances of the blood brain barrier 100 cc. of a 0.5 per cent solution of trypan blue was given via the femoral vein over a 10 to 15 min. interval. After the injection of the dye the cerebral vessels were flushed with 200 to 300 cc. of saline and the animal immediately perfused with 10 per cent formalin for fixation. Following perfusion the cranium was removed and the dura opened. The head with the exposed brain *in situ* was immersed in fresh 10 per cent formalin for 24 hr. at which time the brain was removed intact, sectioned and examined.

Observations were made upon the blood pressure, pulse, respiration and pupil size during and immediately following the injections.

The radiographic densities of similar concentrations and amounts of the various media were compared.

After evaluation of the laboratory data percutaneous carotid angiography was performed on 260 patients using Hypaque as the contrast medium.

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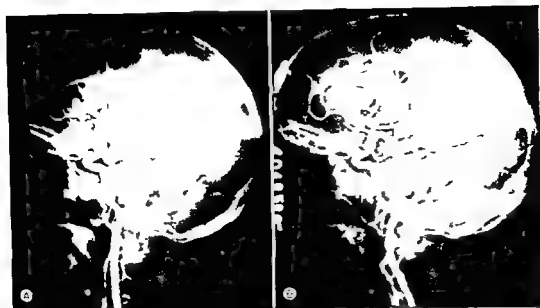


Fig 1

patients were evaluated when the procedure was done under local anesthesia. The patients were questioned regarding pain and discomfort during and immediately following the injections. A majority complained of some paresthesias or dysesthesias over the face and scalp (mainly a burning pain) usually on the side of the injection. This was accentuated when a thrombosis of the internal carotid artery was present and the medium was shunted into the external carotid artery.

Two patients expired within 12 hr following angiography. One a 16 yr old white male was admitted shortly after the onset of a sudden hemiplegia. An angiogram using 2 injections of 6 cc each of 25 per cent Hypaque was normal. The patient expired suddenly 11 hr later. At autopsy a large acute parietal lobe hematoma was found. A second patient a 41 yr old white female died 6 hr after a vertebral angiogram using Hypaque. The autopsy



Fig 2

Initially 6 cc of a 25 per cent solution was used per injection. Eventually a 50 per cent solution was used in all cases in quantities of 10 to 25 cc per injection. (Usually 1 injection of 10 to 12 cc each were used for bilateral angiography.) Either local or sodium pentothal anesthesia was employed in the procedures.

RESULTS

Animal Experiments The immediate effects of rapid injection of Hypaque into the carotid artery of the dog were 1) transient apnea followed by temporary increase in respiration 2) temporary slowing of the pulse 3) temporary dilatation of the pupil on the side of the injection and 4) occasional movements of the facial musculature. These effects were also observed when other media were employed and varied in intensity with the different media and their concentrations.

Animals were given Hypaque in amounts varying up to 4 injections of 20 cc each. In none was there gross staining by trypan blue in the cut sections of the brains. In the brains of the animals receiving Diodrast and Urokon gross staining was present, often limited to the side of the injection. The amount of staining varied with the amount and concentration of contrast material used. Minimal staining was observed in the brains of the animals receiving Renographin and no staining was seen when Thorotrast was used. Engorgement of the blood vessels, particularly in the region of the basal ganglia, was a common finding noted following the use of any of the media provided adequate amounts and concentrations were employed. This effect was most marked in the brains of the animals receiving Diodrast and Urokon.

The radiographic density of 50 per cent Hypaque compared favorably with that of Thorotrast. Because of the necessity for using lesser concentrations of Diodrast, Urokon, and Renographin, the contrast densities of these media were considerably less than either Thorotrast or Hypaque.

Clinical Results Percutaneous carotid angiography was performed on patients in all age groups with satisfactory visualization of the cerebrovascular system. Figure 1 shows a preoperative angiogram of a patient in whom Thorotrast was used compared with a postoperative angiogram on the same patient using Hypaque.

Most of the angiograms were done using 2 injections of 10 to 12 cc each of 50 per cent Hypaque for each side injected. Occasionally, larger amounts were used for better visualization of a lesion. Figure 2 illustrates angiograms using first 10 cc and then 20 cc to more clearly demonstrate the lesion.

No complications have occurred from the use of these larger quantities of Hypaque. The use of larger quantities of contrast medium lends itself to the employment of single exposure technique. When the Fairchild camera or other multiple exposure techniques are used, much smaller amounts give satisfactory studies.

Complications All patients were skin tested with Hypaque prior to angiography and no responses contra indicated the use of the medium. There were no clinical manifestations of allergic responses to Hypaque. This is in agreement with observations made by Lowman *et al*² in their experience with Hypaque in excretory urography.

The effects of the rapid injection of Hypaque into the carotid arteries of

gesia spotty areas of hypalgesia below the level of analgesia and recurrent pain related to analgesic areas.

Ten patients have therefore been selected over the course of the last 3 yrs for study with electrical stimulation in an attempt to further define the changes in sensation which follow this procedure. Only those patients who gave reproducible reports of threshold values for sensations were studied in detail.

METHOD

In the early phase of this study skin stimulation was used. A monopolar electrode with a 1 mm silver ball on the end of a coil spring was moved lightly and slowly over predetermined areas of normal skin. In this manner the threshold values for touch or tingling and for pricking pain were outlined for the arm, thorax, abdomen, and leg. Reproducible results could be obtained at 1 sitting but varied from day to day depending on the humidity, perspiration, and room temperature. Frequencies were varied from 1 to 40 or 100/sec and voltage varied to threshold. Duration of each square wave pulse varied from 0.02 to 2 msec. Only touch and prick thresholds could be measured by this technique. Therefore an insulated electrode with a grounded shield was inserted into the superficial radial nerve of 2 patients after cordotomy with total analgesia to C3 and C5 respectively. Again stimuli of 0.02 to 2 msec duration and frequencies of 1, 10, and 40/sec were used raising the voltage to threshold. A pressure cuff was placed on the arm after touch thresholds were determined and thresholds for pricking pain and finally burning orching pain were determined.

Drugs were given to some patients to determine their effect on these pain threshold studies. Demerol and morphine and 1 V pentothal in dosage sufficient to cause drowsiness were given to 2 patients and 5 to 40 per cent nitrous oxide was given to 5. The threshold studies could be determined with ease and full cooperation at 20 per cent nitrous oxide. Four patients were totally unaware of being given anything but oxygen until they were given 30 per cent nitrous oxide and were unreliable only near 10 per cent nitrous oxide.

DISCUSSION

White and Sweet¹ in their recent classical work on pain state that 10 patients who were tested with electrical skin stimulation in analgesic areas after a clinically successful cordotomy all reported unpleasant or painful sensations. In general we have confirmed their observations in our patients. However in 1 instance the patient reported a sensation of a throb rather than pain when an electric stimulus strong enough to spark to her skin was applied while still in the operating room. Six hours later however she reported pricking painful sensations on skin stimulation. In 2 instances pain was referred to other areas: once from a small analgesic area on the thorax to the hypalgesic ipsilateral axilla and once to a mirror position on the normal right side of the abdomen from a punctate area on the analgesic left side.

Threshold values on the analgesic side exceeded in voltage the normal control side by only 2 to 6 times at a given frequency. In 1 case followed for 18 mo. the values on the side of analgesia were within the range of variability on the normal side.

showed a complete occlusion of the basilar artery with atheromatous plaques. A third patient, a 34 yr old colored female, developed a unilateral facial weakness following angiography. The weakness disappeared after 2 days.

DISCUSSION

Angiographic studies obtained with the use of Hypaque as the contrast medium were of excellent contrast quality. The radiographic density is less than that obtained with Thorotrast, but superior to that obtained with safe concentrations of Diodrast or Urokon. Renographin appears to be a safe medium in lower concentrations although some degree of contrast density is lost in dilution.

In a previous publication we reported angiographic complications using Diodrast or Thorotrast in 5 patients in 215 studies.³ All complications occurred in patients over the age of 60 suspected of having cerebrovascular disease. Fewer complications have occurred with Hypaque. Smolik and Nash⁴ reported a similar experience using Hypaque.

The discomfort to the patient during the injection of Hypaque under local anesthesia can be reduced either by the use of lesser concentrations of the medium or by the use of smaller quantities per injection during multiple exposure technique.

SUMMARY

An experimental and clinical evaluation of Hypaque Sodium as a contrast medium for cerebral angiography shows that it is superior to other media presently in use. Its superiority is based upon its tolerance by the patient at the time of injection, a high degree of radiographic density, and its safety in patients over the age of 50.

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POST CORDOTOMY STUDIES OF PAIN THRESHOLDS*

ROBERT B. KING

Spinothalamic tractotomy has become a well established neurosurgical procedure for the relief of intractable pain. However, reports indicate that from 10 to 30 per cent of these patients are either not totally or permanently relieved of pain by this procedure. These failures have shown a number of disturbing clinical findings including falling and fading levels of anal

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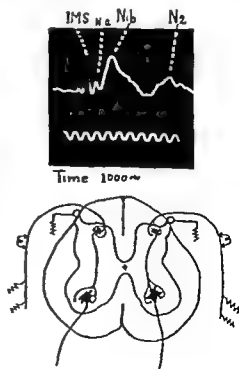
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DECREASE OF SPASTICITY BY PHYSIOLOGICAL DORSAL ROOT RHIZOTOMY*

G M ALSTIN G P MCCOLCH AND F C GRANT

Previous work on the spinal cord of cats and monkeys showed that it was possible to identify and measure the potential derived from afferent terminals (N_1a) within the spinal cord (Fig 1) when a dorsal root was stimulated.¹ One of us (G McC) had previously conceived the idea that spasticity might result from sprouting of new dorsal root afferent terminals within the spinal cord (Fig 2) Utilizing the potential of afferent terminals (N_1a) as a tool we attempted to determine whether or not this was the case in chronically hemisected cats and monkeys. In chronic midthoracic hemisections of the spinal cord where a spastic monoplegia was produced N_1a was increased in amplitude from 10 to 100 per cent on the side of the monoplegia (Fig 3). In addition histologic studies showed that there was an increase of 8 to 58

Fig 1 Bottom Shows diagram of spinal cord at site of stimulation and recording Top Cathode ray photo of cord potential recording response to stimulation of L6 dorsal root from the root entrance zone



Stimulation of the superficial radial nerve using a pressure cuff so as to be able to study C fiber pain gave further findings in 1 case with clinical analgesia to C2. Threshold values were similar to a normal control subject and the patient clearly gave thresholds for tingling, pricking pain, and burning pain. On 1 occasion she complained bitterly of a deep itching pain at the top of the pressure cuff after 31 min. and stimulation at this time for C fibers caused severe burning pain, sweating, nausea, and great anguish.

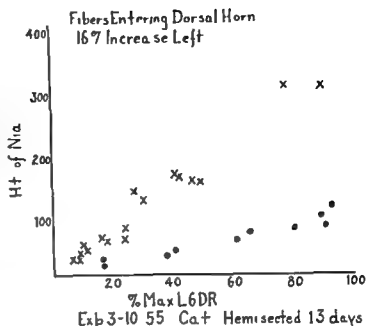
When 20 per cent nitrous oxide was given to these patients 2 observations of note were apparent. In 5 instances where there had been a distinct drop in their level of analgesia to pin prick, the level rose quickly to its highest postoperative level. This observation was made in 1 instance 18 mo. after unilateral high cervical cordotomy in which the level had fallen from C5 to T8 and with 20 per cent nitrous oxide again rose to C5 within 12 min. Despite this dramatic rise in the level of pin prick analgesia there were only minor changes of threshold for prick or electrical skin stimulation. In 2 instances where the analgesic level fell far enough to allow a return of spontaneous pain the patients stated under 20 per cent nitrous oxide their spontaneous pain also disappeared with the ascent of the pin prick analgesia.

Stimulation of the superficial radial nerve under pressure block and 20 per cent nitrous oxide in 1 case showed no evidence of summation at high frequency stimulation of C fibers but thresholds were otherwise not altered. Without the 20 per cent nitrous oxide this patient showed clear evidence of summation in C fiber responses in that threshold values for pain fell approximately 60 per cent at 400/sec. but were not measurably different for this range of frequencies with the nitrous oxide.

These observations suggest 2 possible explanations. It may be that an injured or partially sectioned spinothalamic pathway allows passage of well synchronized electrical stimuli and to a lesser degree the relatively unsynchronized impulses generated by pin prick from hyperalgesic skin areas. In such a circumstance nitrous oxide may interfere with conduction in the region of trauma enough to block the passage of pin prick stimuli but not those due to brief electrical stimulation. An alternate explanation suggests that a pain pathway exists in man inside from the spinothalamic system. It is known that there is no direct spinothalamic system in cats and dogs and very little in monkeys. Even in lower primates Marchi degeneration studies show few fibers entering the thalamus and in cats no apparent sensory deficit follows simple section of the interolateral column of the spinal cord. In these lower forms there is apparently a multisynaptic system or small unmyelinated fiber system for conduction of pain impulses which is dispersed widely through the cord and may even cross from left to right and back across the spinal cord as suggested by the observations of Karpilus and Kraditz² cited by White and Sweet.

The observations on these 10 patients suggest that man may have a system in the spinal cord for relaying a painful stimulus to higher centers in the absence of spinothalamic conduction. The effectiveness of small amounts of nitrous oxide in blocking this system suggests a multisynaptic chain or system and the facility with which highly synchronized electrical impulses may be transmitted across this pathway without the passage of relatively poorly synchronized stimuli (as a pin prick or scratch) strengthens such a concept.

Fig 3 Graph plotting amplitude of N_1 potential against percent of maximal dorsal root spike height. The crosses refer to the chronically hemisected side and the dots to the control side (acutely hemisected)



tive procedure for permanently stopping spasticity in total paraplegia.⁴ However when there is only partial spinal cord section with resulting paraparesis and spasticity selective dorsal rhizotomy may prove extremely effective. When remaining downstream fibers have regenerated beyond the site of injury and reach their former destination in the interneuronal pools involved in motor movement there is unavailable space left for them to reestablish synaptic endings. One way of permitting them to reestablish synaptic connections is to temporarily block and cause degeneration of the dorsal root afferents which have proliferated and assumed control over spinal cord function. Thus a selective dorsal root rhizotomy would decrease spasticity and provide the mechanism by which downstream fibers if they have regenerated past the site of injury may return to the point of producing effective motor movement. The ultimate result of selective dorsal root rhizotomy probably depends on a competitive sprouting between remaining adjacent dorsal roots and corticospinal fibers.

We have now operated on 6 patients who have shown severe spasticity with various degrees of mass reflexes. It was with the anticipation that the procedure would decrease the severe flexor spasms and mass reflexes that the operation was carried out. However 3 of these patients had slight movement of the leg foot and toes. It was felt that if their downstream afferents were intact the operative procedure itself would provide optimal conditions for improved motor function. Obvious difficulties are apparent in this line of attack. First one is unsure as to how much remains or has regenerated in the way of downstream pathways past the site of injury. Another and more serious problem in this type of procedure is that unless patients are seen fairly early in the course of their disease one will encounter severe contractures of the adductors hamstrings and achilles tendons. Then the procedure although it may be markedly helpful faces the physical opposition of the tendon contractures. Finally an extremely important factor has proven to be intelligent motivation. Unless a patient is willing to intensively practice to improve motor movement the operation is unlikely to be successful.

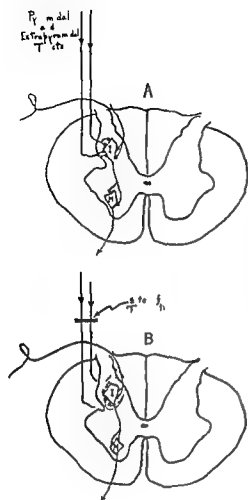


Fig 2 A Normal relationship of downstream pathways and dorsal root fibers ending on interneurons B Sprouting of new dorsal root afferents following cord injury

per cent in the number of afferent fibers from the dorsal root entering the dorsal horn on the side of the chronic hemisection at the site of stimulation. This provided evidence for a hypothesis of the development of spasticity. If the spinal cord is damaged severely enough to interrupt downstream axons these fibers degenerate from the boutons upward toward the site of injury. Then within a period of 1 to 2 wks there is a gradual sprouting of new afferent terminals from dorsal roots. These terminals end predominantly on interneuronal pools and form synaptic connections in positions left vacant by degenerated downstream terminals. Once synapses have been established by the newly sprouted afferents the effective stimulus over these dorsal roots is greatly increased from the normal situation. This provides the necessary mechanism for hyperactivity of the reflexes following damage to downstream pyramidal and extrapyramidal systems.

With this physiologic background in mind it seemed that one way to decrease spasticity and pain associated with mass reflexes in the human would be to limit the afferent input to the interneuronal pools. This is best done by interruption of selected dorsal roots and their freshly sprouted afferents (Fig 2). However if a few dorsal root afferents are cut there are always other dorsal root afferents capable of sprouting from adjacent levels and which according to the evidence of Ito and Chambers³ may cover a distance of as much as 6 segments in their attempts to establish synaptic connections. This suggests why rhizotomy of only 3 or 4 dorsal roots remains an ineffec-

CLINICAL AND EXPERIMENTAL STUDIES ASSOCIATED WITH ELECTROLYSIS OF LISSAUER'S TRACT*

ROBERT W. RAND, ERNEST J. PENKA AND W. EUGENE STERN

In 1912 and 1913 Hyndman and coworkers^{1, 2} focused attention on Lissauer's tract in man by demonstrating that its destruction in the thoracic cord resulted in ipsilateral analgesia and thermesthesia in a band 3 to 5 segments wide. Such procedures in the cervical cord did not give similar results. Stimulated by the potentialities of these results a program employing a technique of electrolysis of this and other spinal cord tracts was commenced.³

METHOD

Five cats and 2 monkeys underwent laminectomies using general nembutal anesthesia and sterile conditions. Neither wound nor meningeal infection occurred. In this first series of experiments unipolar direct low amperage current (0.5 to 1.0 ma) needle electrolysis for 15 to 45 sec. was employed in different combinations to produce multiple lesions in Lissauer's tract. In the postoperative period neurological examinations and cinematography assessed the neurologic changes. The cervical and upper thoracic cords were serially sectioned and stained alternately by hematoxylineosin and Smith's myelin methods 3 wks to 3 mo after the Lissauer tractolysis.

In addition multiple lesions in Lissauer's tract have been made during the course of thoracic cord surgery on 2 cancer patients using 0.5 ma for 30 sec for each of 6 and 9 lesions respectively.

RESULTS

Cat 1 Three lesions were produced on the left side at 1 mm depths in the Lissauer tract of the C6 and C7 cord segments using 1.0 ma for 30 sec. at C8 and T1 levels there were similar lesions with 0.5 ma for 15 sec. On the right 3 lesions were made in this tract in the cord segment C6-7 and T1 using 0.5 ma for 30 sec. Immediately after operation analgesia was present in the left forearm and paw with marked hypalgesia on the right in similar areas. The cat could move all extremities but was unable to walk. After 3 wks the animal could walk in a slinking manner with truncal awkwardness. Seven weeks postoperatively almost normal climbing and running was possible. There was marked hypalgesia of the left forearm and paw and moderate hypalgesia of the right. Motor function improved steadily but the sensory deficit persisted. No motor impairment was noted when sacrificed 3 mo after operation.

A wedge shaped scar replaced the entire Lissauer tract over the C6 segment on the left and extended slightly into the upper dorsal spinocerebellar tract and the posterior column. On the right small intermittently spaced areas of gliosis were noted in Lissauer's tract. There were 2 small areas of myelomalacia in the base of each posterior horn. Similar findings were noted in the C7 cord segment. No distinct lesions in Lissauer's tract were found at the

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Our initial 3 cases were of paraplegia following spinal transection. Extensive dorsal rhizotomy (L1-S1) relieved the pain associated with the spasms but some of the spasticity returned in 3 to 6 mo. Our fourth patient (A S) was completely blind following a tubercular arachnoiditis. He had severe paraparesis hamstring contractures with pain and spasticity. An extensive dorsal rhizotomy (L2-S1) relieved a great deal of the spasticity and also his pain but he regained only slight motor power in the feet and toes. Another patient (A T) had sustained a severe head injury with resulting spastic paraparesis and weakness of the right arm. In addition there was mental impairment following the injury. Dorsal rhizotomy was again ineffective (L3-L5-S1) in producing more than 15 per cent motor recovery in the feet and toes. His spasticity was decreased temporarily but he was never able to overcome the tendon contractures of the hamstrings and seemed to possess no strong motivation to improve.

Our sixth case was that of a 30 yr old white woman with marked paraparesis severe extensor and adductor spasms but no tendon contractures. She had been shot at T10 with resulting cord contusion. Prior to operation she was unable to stand except when supported by 2 people. Dorsal rhizotomy of L2-L4 and S1 was done bilaterally, 6 mo after the injury. Six months after the rhizotomy she can walk unassisted with the use of a cane. Her extensor spasms were stopped and motor power continues to increase. She has a great deal of motivation and has exercised faithfully in attempts to help herself.

In conclusion selective dorsal rhizotomy seems worthwhile in certain cases of paraparesis when spasticity is an incapacitating factor and where lack of tendon contractures and a strongly motivated patient favor recovery.

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ficial Lissauer tract lesions associated with localized arachnoiditis were slightly misplaced medially in the lowest cord segments. Varying degrees of myelomalacia was observed throughout most of the transverse gray similar to Cat 1. The lateral aspect of the anterior horns was involved in some segments as well as the base of the posterior columns.

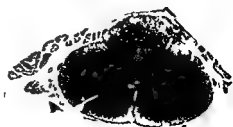
The weakness exhibited in the right paw was certainly due to anterior horn cell involvement. The hypalgesia was undoubtedly the result of myelomalacia of the transverse gray and the base of the posterior horns rather than lesions in Lissauer's tract. The infarctions were strikingly similar to Cat 3 and 4 where there was also a mild subarachnoid hemorrhage along a posterior root.

Monkey 1 Ten lesions were produced 1 mm deep in Lissauer's tract on the right with 0.5 ma for 15 sec from the sixth cervical through the first thoracic cord segments. The gross and fine movements and coordination of the extremities during climbing, jumping and eating were normal postoperatively except for some initial swaying of the pelvis for 1 wk. Many attempts to test sensory dysfunction failed. However while undergoing slow anesthesia prior to necropsy examination 6 wks after operation a definite analgesia of the entire ulnar aspect of the right forearm and a profound hypalgesia in the ulnar distribution of the right hand and fourth and fifth fingers were observed. In contrast quick violent retraction of the other extremities occurred during forceful painful stimulation. From the mid portion of C6 through the upper T1 cord segments the right Lissauer tract was replaced at the site of the lesions by either a dense wedge shaped cicatrix extending to the substantia gelatinosa or lytic area conforming to the Lissauer tract (Fig. 1). Although quite limited to the Lissauer area in C6-7 and T1 levels it spread slightly into the posterior column and the upper portion of the dorsal spinocerebellar tract at the C8 cord segment. There was an area of myelomalacia in the base of the left posterior horn at T1 levels. At the T2-3 cord levels myelomalacia appeared in the transverse gray. In places this malacia extended slightly into the left posterior column and the lateral funiculi.

The analgesia and hypalgesia of the right forearm and hand were undoubtedly due to interruption of the right Lissauer tract. No other pathological changes from C6 through T1 cord segments were present. The central myelomalacia of T2-3 levels was well below the site of the last lesion and was not associated with significant arachnoiditis. This malacia probably did not contribute to the neurologic sensory deficit of the right upper extremity.

Monkey 2 Fifteen lesions 1 mm deep were made in the right Lissauer tract from C5 through T1 cord segments with 0.5 ma for 30 sec. Postopera-

Fig. 1. Electrolytic lesion in the right Lissauer tract of monkey 1 causing its complete destruction at the C8-T1 level without other significant pathological changes.



C8 level. Initially myelomalacia at the base of the left posterior horn extended into upper portions of the anterior horn and adjacent white substance. Thereafter it spread to involve the transverse gray. In the T1 cord segment several superficial lesions in the Lissauer tract were present. Similar degenerative changes in the entire transverse gray continued including the lateral funiculus medially.

In spite of early motor disturbances this animal eventually ran, climbed and jumped normally. Apparently the hypalgesia in the forearms and paws resulted from a combination of involvement of the transverse gray, posterior horn and Lissauer's tract.

Cat 2. Three lesions each $\frac{3}{4}$ mm in depth were made in the C5, 6, 7, 8 and T1 cord segments employing 1.0 ma for 30 sec. The animal died of nembutal intoxication. Some of the lesions were found to be misplaced lateral to the Lissauer tract. Thereafter magnifying glasses resulted in greater accuracy of needle placement.

Cat 3. Using 0.5 ma for 30 sec at a depth of 1 mm a total of 15 lesions were produced along Lissauer's tract from C5 through T1 cord segments on the right. A small subarachnoid hemorrhage occurred at the left C6 level. Although there was poverty of movement of the right forearm, and the animal walked in a stalking manner for 2 wks thereafter it ran, jumped and climbed without any apparent paresis or loss of position sense. During this period analgesia of the entire right upper extremity and moderate hypalgesia of the left to the elbow was noted. The right-sided analgesia gradually changed to a marked hypalgesia while normal sensation returned on the left. Incomplete lesions along Lissauer's tract associated with the usual amount of arachnoiditis were found 6 wks after operation. Varying degrees of myelomalacia of a considerable portion of the transverse gray at each cord segment was observed. The anterior commissure was not completely involved even in the areas of most marked myelomalacia. In a few areas demyelination of the lateral funiculus was present medially.

One would have expected the bilateral hypalgesia to be persistent if the transverse gray myelomalacia were totally responsible. Therefore it is suggestive that the Lissauer tract lesions, even though superficial, contributed to the right upper extremity hypalgesia.

Cat 4. This animal is included as a control. A small local subarachnoid hemorrhage over the right C6 root occurred during the exposure as it had in Cat 3. No Lissauer tract lesions were made. The animal developed marked paresis of the right forepaw and bilateral analgesia to the elbows. The symptoms persisted in lessened degree over the 3 wk survival period. An area of severe myelomalacia at C6-7 involved the lateral portions of the right anterior horn as well as the base of the right posterior horn and the anterior commissure in some areas.

Cat 5. Five left Lissauer tract lesions were made 0.5 mm deep in the cord segments C5, 6 and 7, four in C8 and 3 in T1 employing 0.5 ma for 15 sec. A small subarachnoid hemorrhage occurred at the right C6-7 level. There was marked paresis of the right paw and questionable hypalgesia which disappeared in 3 wks, permitting normal motor activity. Although difficult to test and evaluate at first due to uncooperation, adequate examination later disclosed definite hypalgesia of the left forearm and paw, being most marked distally, which persisted to time of autopsy 1 mo after surgery. The super

ficial Lissauer tract lesions associated with localized arachnoiditis were slightly misplaced medially in the lowest cord segments. Varying degrees of myelomalacia was observed throughout most of the transverse gray similar to C 4. The lateral aspect of the anterior horns was involved in some segments as well as the base of the posterior columns.

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Fig. 1. Electrolytic lesion in the right Lissauer tract of monkey 1 causing its complete destruction at the C8-T1 level without other significant pathological changes.



tively the animal had severe weakness of the forearms and hands especially on the right. This improved slightly. Sensation to pain was appreciated in the left upper extremity and hand and in the right thumb and index finger. An algesia of the ulnar aspect of the right hand and forearm was present. A severe paraparesis in extension progressed over the course of 2 wks into a complete paraplegia in flexion below the T2 cord segment. The sensory findings in the upper extremities remained essentially unchanged at the time of autopsy 3 wks after operation. Multiple infarctions of the right Lissauer tract from C6 through C8-T1 levels extended into the lateral half of the ipsilateral transverse gray and upper portions of the anterior horn. Below T1-2 levels the entire central gray was completely destroyed with demyelination extending well into the lateral funiculi.

Rather than cicatrix lesions of the Lissauer tract these lesions were clearly vascular in type but apparently did result in the analgesia over the C7 through T1 dermatomes on the right. Below T1-2 levels the severe central infarction caused the eventual complete paraplegia in flexion.

Patient 1 Nine lesions were placed in the right Lissauer tract over the T2 3rd and 4th cord segments at a 1 mm depth using 0.5 ma current for 30 sec. No areas of hypalgesia developed. There was no paresis of the legs. Five days later posterior rhizotomy of T2 through T5 was carried out to relieve the intercostal pain secondary to bronchogenic carcinoma.

Patient 2 Six lesions were made in the right Lissauer tract at T3-4 levels during the course of a right interlateral cordotomy at T2 for the relief of unilateral left leg pain due to metastatic carcinoma invasion of the left lumbosacral plexus. The lesions were 1.5 mm deep and produced by 0.5 ma for 30 sec. Again no sensory changes occurred attributable to the Lissauer tract lesions. Neither weakness of the right or left leg nor sensory disturbance of the right lower extremity was observed.

SUMMARY AND CONCLUSIONS

The electrolytic technique herein described can produce discrete multiple lesions in Lissauer's tract.

In the absence of other associated spinal cord changes the resultant analgesia and hypalgesia is probably related to these lesions in Lissauer's tract (monkey 1).

Associated distant vascular myelomalacia can occur with this technique due either to subarachnoid hemorrhage from tearing of a small posterior radicular artery or electrocoagulation of penetrating vessels in the posterior root.

The application of this electrolytic technique had as yet not been associated with persistent analgesia in man.

Further modification of this electrolytic technique is being undertaken in an effort to avoid distant vascular infarction in the spinal cord.

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SELECTIVE SACRAL NEURECTOMY FOR HUNNER'S ULCER WITH NEUROPHYSIOLOGIC OBSERVATIONS*

ROBERT D. WHITTIED PAUL W. MYERS WILLIAM A. MEINER
AND EDWIN C. CAMBEL†

The treatment of chronic interstitial cystitis has been difficult and discouraging since Hunner first described the condition in 1911. Over the years it became a clinical entity, sometimes unrecognized because of the obscurity of the pathology. Hunner in fact called the condition "ulcer." Today the disease is known as chronic interstitial cystitis or Hunner's ulcer.

Over 95 per cent of the cases occur in females who complain of frequency and pain on urination, usually occurring in irregular cycles. Cystoscopic examination shows linear cracks in the bladder mucosa at the dome that bleed on the slightest provocation. Surrounding this cracked mucosa is a radiating network of congested vessels.

Meredith Campbell in his text of urology states: "no other condition except cancer or tuberculosis of the bladder can cause such intractable or severe pain. As in any disease of obscure etiology, treatment is varied."

Hunner first recommended partial suprapubic cystectomy. This radical procedure has been followed by bladder distention, silver nitrate irrigations, radium, vitamin E, estrogens, presacral neurectomy and frequent fulguration. It was clear that any approach to permanent relief of pain and frequency for these unfortunate individuals lay in a new direction.

Meirowsky¹ first performed selective sacral neurectomy in 2 patients for the treatment of Hunner's ulcer. Personal communication with him prior to the publication of his results aroused our interest in the problem.

Our series consists of 6 patients who were studied and operated upon between December 1955 and May 1956. Five of these had typical Hunner's ulcers. All of them were female, ranging in age from 35 to 69 with an average age of 53.2 years and a median age of 52 years. Their symptoms had been present between 5 and 12 yr, with an average of 9.2 yr and a median of 8.5 yr. All had severe pain. All had had fulguration, silver nitrate instillation, dilatation of the bladder and sulfonamides or various combinations of these. Two had had gross hematuria: 1 microscopic, 2 none. In every instance the ulcer had been observed cystoscopically.

The sixth was a 51-year-old male who had transurethral resection of a carcinoma of the bladder 3½ yr previously. An ulcer resulted which had many of the characteristics of Hunner's ulcer—appearance, severe pain, frequency of urination and gross hematuria. For these reasons he was considered a suitable candidate. Unfortunately, massive recurrence of his primary tumor prevented any lasting benefit. Nonetheless the observations made before operation contribute their part to this study and are therefore included.

METHOD

Preoperative Observations. Prior to operation, selective blocks of the sacral nerves were made under radiographic control. Bladder capacity was

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†Deceased.

Table 1 Observations at the Time of Block

PATIENT	NERVES BLOCKED	BLADDER CAPACITY (CUBIC CENTIMETERS)		BLADDER PRESSURE mm H ₂ O	
		BEFORE	AFTER	BEFORE	AFTER
1 (BH)	S3	45	170	NOT MEASURED	
2 (HI)	S3	90	200	600	130
3 (MI)	S3	175	200	320	210
4 (CA)	S3	290	380	170	160
5 (RS)	S3	20	60	210	220
II (MR)	S3	30	50	360	100
	S2	50	60	350	580

determined before and after block and, the intravesical pressure at maximum distention was also established. The results are recorded in Table 1.

Operative Exposure. Midline incision was made over the sacrum. The musculature was stripped back beyond the sacral crests bilaterally. The sacral foramina were identified by reference to the spinous process of the 5th lumbar vertebra. The second and third sacral foramina were unroofed; the nerves (anterior primary rami) freed and, in 3 cases the second and third sacral nerves stimulated in turn while cystometrographic observations were made. A member of this group estimated by digital examination the contractions of the anal sphincter and of the levatores ani. The third sacral nerves were then sectioned in 5 cases; the second sacral nerves were ligated and the distal segment injected with 95 per cent alcohol in Case #6.

Observations at Operation. In the later cases 3 in number the results of stimulation were recorded (Table 2). In 1 instance the second and third sacral nerves contributed to bladder innervation; in the other 2 the third sacral nerve alone was of importance.

RESULTS

As shown in Tables 3 and 4 material subjective and objective improvement was secured while the undesirable effects were slight. The relief of pain and decrease in frequency of urination were striking. Four ulcers healed. This continued to have recurrent microscopic hematuria. Case #6 who had recurrent carcinoma is excluded.

Table 2 Observations at Operation

PATIENT	NERVES STIMULATED BILATERALLY	CHANGE IN BLADDER PRESSURE (mm H ₂ O)		RECTAL SPHINCTER MASS	LEVATOR ANI CONTRACTION	NERVES DIVIDED BILATERALLY
		BEFORE	AFTER			
II (HI)	S2	120	150	Marked		
	S3	120	180	Slight		S3
I (CA)	S2	None		Marked	None	
	S3	200	600	None	Moderate	S3
5 (RS)	S2	None		None	Moderate	
	S3	20	100+	Marked	None	S3

No stimulation in Cases #1, 3 and 4

Table 3 Post Operative Results (Bladder)

PATIENT	NERVES DIVULSED	BLADDER CAPACITY (CUBIC CENTIMETERS)		FREQUENCY		HEALING OF ULCER
		PRIOR	POSTOP	PRIOR	POSTOP	
1 (BH)	S3	40	160	10 min	1 hr	Yes
2 (HI)	S3	30	300	20 min	3 hr	Yes
3 (MP)	S3	10	200	1 min	3 hr	Healed
4 (CA)	S3	200	300	1 hr	6 hr	Yes
(RS)	S3	-	-	10 min	20 hr	Yes

Note: Patient #6 (MR) had recurrence of carcinoma requiring total cystectomy

Table 4 Post Operative Deficits

PATIENT	RECTAL SPHINCTER TONE—% OF NORMAL	RECTAL INCONTINENCE	NEUROLOGIC DEFICIT
1 (BH)	75%	None	None
2 (HI)	75%	None	Hypalgesia lateral left thigh
3 (MP)	75%	None	Hypalgesia S3 absent gluteal reflexes
4 (CA)	50%	None	Feet numbness (subjective)
(RS)	75%	None	Absent ankle jerks
6 (MR)	75%	None	Hypalgesia S2 S3

Recurrent carcinoma

SUMMARY

1 Observations upon 5 patients suffering from typical Hunner's ulcers and upon 1 with a similar ulcer resulting from carcinoma of the bladder are recorded

2 In all our cases the third sacral nerves contributed to bladder detrusor activity

3 In 2 cases the second sacral nerves did not contribute to bladder detrusor activity in 2 they did and in 2 our observations do not provide any information

4 In all 5 cases of true Hunner's ulcer bilateral section of the third sacral nerves produced subjective relief of symptoms and objective evidence of improvement

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	{ S2	50	60	350	580

determined before and after block and the intravesical pressure at maximum distention was also established. The results are recorded in Table 1.

Operative Exposure Midline incision was made over the sacrum. The musculature was stripped back beyond the sacral crests bilaterally. The sacral foramina were identified by reference to the spinous process of the 5th lumbar vertebra. The second and third sacral foramina were unroofed; the nerves (anterior primary rami) freed and in 3 cases the second and third sacral nerves stimulated in turn while cystometrographic observations were made. A member of this group estimated by digital examination the contractions of the anal sphincter and of the levatores ani. The third sacral nerves were then sectioned in 5 cases; the second sacral nerves were ligated and the distal segment injected with 95 per cent alcohol in Case #6.

Observations at Operation In the later cases, 3 in number, the results of stimulation were recorded (Table 2). In 1 instance the second and third sacral nerves contributed to bladder innervation; in the other 2 the third sacral nerve alone was of importance.

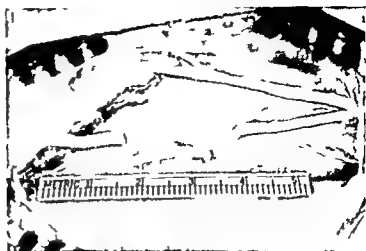
RESULTS

As shown in Tables 3 and 4 material subjective and objective improvement was secured while the undesirable effects were slight. The relief of pain and decrease in frequency of urination were striking. Four ulcers healed, 1 has continued to have recurrent microscopic hematuria. Case #6 who had recurrent carcinoma is excluded.

Table 2 Observations at Operation

PATIENT	NERVES STIMULATED BILATERALLY	CHANGE IN BLADDER PRESSURE (mm H ₂ O)	RECTAL SPHINCTER SYSTEM	LEVATOR ANI CONTRACTION	NERVES DIVIDED BILATERALLY
2 (H I)	{ S2	120-150	Marked		
	{ S3	120-380	Slight		S3
1 (C A)	{ S2	None	Marked	None	
	{ S3	200-400	None	Moderate	S3
5 (R S)	{ S2	None	None	Moderate	
	{ S3	210-600+	Marked	None	S3

No stimulation in Cases #1, 3 and 6



Figs 1, 2, and 3 amplify the text. After introduction into the nerve ends atraumatic sutures are threaded on a long needle for passage through the tube and are then removed.



strength is poor and experience has shown that a reinforcing matrix is advantageous. Impregnation of the filter material on #58 stainless steel mesh overcomes the problem of fragility but sacrifices flexibility.

Between the third and fourth week after implantation the unreinforced tubes were contained within gossamer thin sheaths of opalescent tissue which were loosely adherent to the tissue bed. A slightly denser fibrous coat surrounded the reinforced tubes. Within the tubes a nonadherent continu-

THE SURGICAL APPLICATION OF MONOMOLECULAR FILTERS (MILLIPORE) TO BRIDGE GAPS IN PERIPHERAL NERVES AND TO PREVENT NEUROMA FORMATION*

JAMES B. CAMPBELL AND C. ANDREW L. BASSITT

The problem of inducing orderly regeneration across an irreducible gap in a peripheral nerve has been approached by grafting¹ an isthmus following staged lengthening,² insertion of a filament scaffold within a vein graft,³ and securing of the proximal and distal stumps within tubes.⁴ The reported limitations of these techniques prompted a search for an ideal conduit within which continuity could be restored by regeneration. Such a conduit should be inert in tissues, permit free passage of extracellular nutrient fluids and at the same time deny access to cells. When the *in vivo* properties of a monomolecular cellulose filter (Millipore HA formulation) were announced by Algire,⁵ these qualifications appeared to be fulfilled.

METHOD

Initially sheets of moistened Millipore were rolled over a glass rod into tubes 6 cm. in length and 3 to 4 mm. in diameter. This form was maintained by a narrow line of bonding with Celvol or Glyptal. The process could be likened to fabricating a cigarette without tobacco. However, the incidence of rupture of tubes made reinforcement of the filter material desirable. Therefore cylinders were fabricated from #58 stainless steel mesh sheets impregnated with Millipore material.

The tibial division of the feline sciatic nerve was isolated and severed. In 1 series of cats the proximal and distal nerve ends were introduced into tubes of Millipore for a distance of 1 cm. A gap of 2.5 cm. in the nerve was maintained by suturing the proximal and distal segments to the muscle bed or to the tube (Fig. 1, 2, 3). In the initial phases of the experiment a sling stitch between the proximal and distal segments prevented their withdrawal from the tube. In a second group of animals the proximal and distal nerve segments were introduced into closely fitted blind end socks of Millipore. Because of discrepancies in the diameter of the tubes and nerves tube ends were crimped with silver clips to create a snug fit and prevent ingress of scar tissue. Between the third and fourth postoperative week the filter was removed and the nerve excised for histological study. However, in 1 cat in which the gap had been satisfactorily bridged at 4 wks. the reuniting nerve was returned to its tissue bed for another 6 wks. after removal of the filter. In a control series the surgery was identical except that Millipore tubes or socks were not applied.

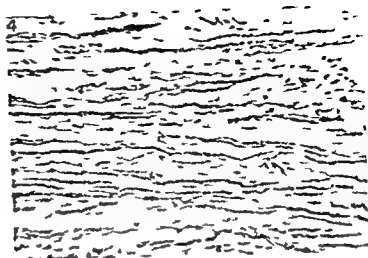
RESULTS

Millipore is brittle. Saturation with water increases flexibility to the extent that careful manipulation becomes possible. However, at best the tensile

From the Departments of Neurological and Orthopedic Surgery, Columbia University College of Physicians and Surgeons and The New York Orthopaedic Hospital, Columbia Presbyterian Medical Center, New York, New York. Supported by grants from the Department of the Army, Office of the Surgeon General (Contract No. DA 19-007 MD 514), Playtex Park Research Institute, and United Cerebral Palsy Associations, Inc.

Millipore supplied by Mr. John Bush, President, Millipore Filter Corporation, Watertown 72, Massachusetts.

Fig 4 Bodian (X400) Longitudinal section of the distal nerve segment at 4 wks



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Fig 5 Mahon (X900) Longitudinal section of 2.5 cm tissue bridge at 10 wks

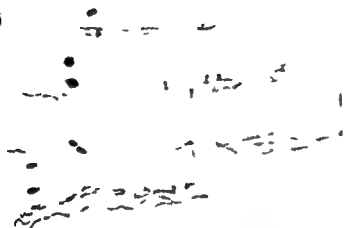
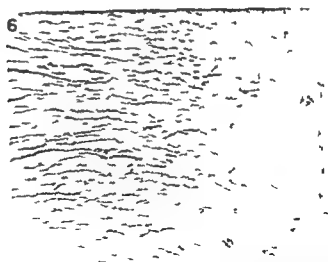


Fig 6 Bodian (X1000) Longitudinal section of proximal nerve stump encased in a filter sock for 4 wks



From these data it seems that during the initial phases of regeneration cells from the circumference of the proximal nerve migrate distally using the walls of the tube as a substrate. In most soft tissues nutrition by diffusion can occur over a distance of 1 mm. It is therefore entirely possible that a layer of neural tissue 1 mm thick can build up on the inner wall of the tube completely independent of blood supply merely by drawing

ous firm opaque cord of tissue united the proximal and distal segments. A striking contrast was provided by the control group and those animals in which the tubes were ruptured. In both instances heavily vascularized bulbous neuromata had formed which were densely adherent to the tissue bed. No tendency to bridge was noted in the gaps.

In the 3 to 4 wk specimens there was histologic evidence of axons enveloped in parallel Schwann tubules extending the entire length of the 2.5 cm gap. There was no evidence of myelin formation. The longitudinal parallelism of fibers in the tissue bridge was a striking feature. The sole evidence of disorganization was found about the sling sutures where a Schwannoma foreign body reaction prevailed. However, in tissues adjacent to the filter material no foreign body response had been evoked. Study of serial longitudinal sections gave evidence that tissue at the periphery of the regenerated cord contained greater numbers of axons than did the central areas. At 3 to 4 wks it was noted that the greater the distance from the proximal stump the fewer the axons. In spite of these observations fibers had spanned the 2.5 cm gap and were observed entering the distal segment of nerve.

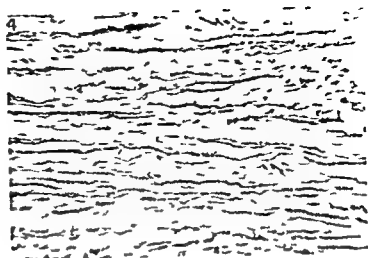
A firm cord of tissue united the nerve segments in the animal in which the unsheathed bridge was returned to its bed 4 wks after the initial procedure. At operation 6 wks later the bridge was easily dissected free by dividing a thin overlying film of tissue. The stimulation of transection caused vigorous contractions of the gastrocnemius muscle as the major fascicles were cut 2 cm distal to the original lower level of sectioning. The color of the cross section and fascicle diameters of the distal nerve were normal. Mature axons in uniform numbers were found throughout the area of bridging and could be traced into the distal stump (Fig. 4). Reorganization of the distal Schwann tubules by axons extended throughout the entire distance of the section. Mahon stains indicated the presence of large quantities of myelin throughout the area of the gap (Fig. 5). In the distal segment fewer myelin deposits were found.

Covering of proximal and distal stumps with blind Millipore socks prevented neuroma formation. At 4 wks after operation the Millipore material again had evoked a minimal host response. Within the filter sock a firm opaque nonadherent nerve end was found its contours accurately reflecting the inner topography of its prison. Microscopic examination of stained sections again demonstrated orderly, parallel axons projecting from the severed proximal nerve end (Fig. 6). Schwann cell proliferation was not as prevalent as in the section taken from the nerve gaps. Continuity was found to have been interrupted in two defective blind end socks. In areas where rents had occurred in the Millipore material invading connective tissue elements created a picture typical of neuroma formation.

DISCUSSION

The H A formulation of monomolecular cellulose filter material can be thought of in simplified terms as a flattened honeycomb with 1 mu openings. The foregoing data suggest that the porosity of this particular plastic creates a favorable nutritional and substrate environment for peripheral nerve regeneration. The material is most inert in tissues, affords a scaffold for regenerating neural elements and shields them from competition with mesenchymal cells arising from the operative bed.

Fig 4 Bodian (X400) Longitudinal section of the distal nerve segment at 4 wks



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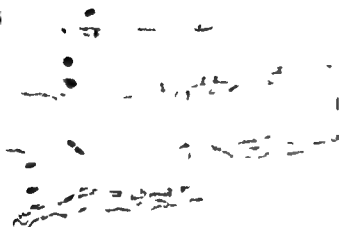
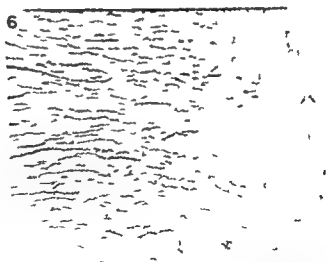


Fig 5 Mahon (X900) Longitudinal section of 2.5 cm tissue bridge at 10 wks

Fig 6 Bodian (X100) Longitudinal section of proximal nerve stump encased in a filter sock for 4 wks



From these data it seems that during the initial phases of regeneration cells from the circumference of the proximal nerve migrate distally using the walls of the tube as a substrate. In most soft tissues nutrition by diffusion can occur over a distance of 1 mm. It is therefore entirely possible that a layer of neural tissue 1 mm thick can build up on the inner wall of the tube completely independent of blood supply merely by drawing

nutrition from diffusing tissue fluids. These factors probably account for the picture of greater tissue maturity seen at the circumference of the bridge at 3 to 4 wks. In seeking reasons for the striking orderly parallelism of the regenerative picture it is interesting to speculate on the possible inductive role of the tube wall.

Garrity⁴ was able to limit neuroma formation by placing the severed ends of peripheral nerve within plastic and rubber tubes, but was unable to achieve regeneration. The fact that mechanical rupture of the walls of the tubes or blind socks was invariably associated with neuroma formation emphasizes the importance of shielding the regenerating axons from outside cells. The Schwannoma like formation in the region of the silk is provisionally accounted for on the basis of foreign body irritation. In more recent experiments the sling suture has been discarded. Fixation sutures passing through the perineurium proximal and distal portions of the tubes have afforded adequate stabilization.

The limited data indicate that the rate of regeneration is consistent with the general concepts of others. Further experimentation utilizing bioelectric techniques will be necessary before definite statements can be made concerning rate of regeneration. The preliminary findings of these studies demand that further work determine the tissue toxicity of Millipore and the functional adequacy of neural regeneration in long term animals. Additional effort must be directed to the technical aspects of tube and sock production. Millipore filter material unreinforced is brittle when dry, friable when wet and generally difficult to handle. Clinical application of the technique must ultimately wait for the development of a suitable reinforcing matrix. Recent trials with woven nylon tubes show promise. It is hoped that this method for controlled neural regeneration may eventually find application in man as an aid in reestablishing neural continuity, reducing scar formation at suture lines and preventing neuroma formation in severed nerves.

CONCLUSIONS

1 Preliminary results of experiments using Millipore filter tubes to achieve bridging of long gaps in peripheral nerves are presented.

2 Regeneration of Schwann tubule encased axons has occurred in an orderly fashion over a distance of 2.5 cm during a 3 to 4 wk interval following operation.

3 The application of snugly fitted blind socks of Millipore to nerve stumps has prevented neuroma formation in short term experiments.

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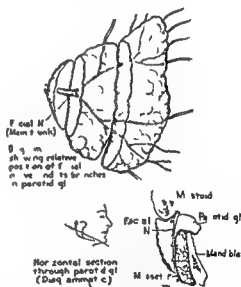
SPONTANEOUS RETURN OF FUNCTION FOLLOWING SURGICAL RESECTION OF THE SEVENTH NERVE FOR PAROTID TUMORS*

HAYS MARTIN AND JAMES I. HEISLER

The purpose of this preliminary report is to briefly present clinical evidence which shows that following surgical section and sacrifice of a considerable segment of the seventh cranial nerve (facial) there can be spontaneous recovery of function in a fair percentage of cases without resort to nerve grafting or other neurotrophly.

The clinical basis for this report is an analysis of a consecutive series of 150 operative cases of malignant tumors of the parotid gland seen on the Head and Neck Service of Memorial Hospital during the period 1919 to 1953 inclusive. Incidental to surgical removal of the tumors 10 patients (27 per cent) had a deliberate section of the 7th cranial nerve and excision of a segment of between 2.5 and 5 cm. of the main trunk of the nerve and its dividing plexus (Fig. 1).

Fig. 1 Diagrammatic representation of the relationship between the 7th cranial nerve (Facial) and the parotid gland



Of the 40 patients with such sacrifice of the nerve 28 were considered to be determinate. The remaining 12 indeterminate patients (all with cancer of the parotid) had either rapid and massive local recurrences with gross invasion of the facial musculature or suffered generalized dissemination of cancer. None of the latter 12 patients lived long enough to establish whether there would eventually have been any return of function.

The noteworthy observation which we wish to place on record is that at least 7 patients (or 25 per cent) of the 28 determinate cases had a fair degree of return of function in the paralyzed facial musculature without nerve grafting or any other reparative operation.

In Table I there is given a brief resume of the 7 cases of spontaneous recovery. The patients ranging in age from 16 to 63 yr. were all operated upon during a 5 year period (1919 to 1953 inclusive). As is the routine in our clinic the main trunk of the 7th nerve had been exposed as a preliminary step of the operation. In all instances the decision to section the main trunk and to sacrifice the nerve was made only after it had been clearly

*From the Head and Neck Service, Memorial Hospital, New York, N.Y.

Table No 1 Pertinent data concerning the 7 patients in whom spontaneous return of function occurred following excision of a segment of the 7th cranial nerve (Facial) incident to surgery for parotid tumor

PATIENT	AGE	SEX	MINIMUM LENGTH OF RESECTED SEGMENT (CM)	DIAGNOSIS	FIRST RETURN OF FUNCTION (MO)
N B	28	F	3	Mucoepidermoid Carcinoma	8
I R	16	F	4	Adenocarcinoma	6
R T	63	M	5	Epidermoid Carcinoma	6
J A	37	M	3	Recurrent Mixed Tumor	10
M I	26	F	2.5	Mucoepidermoid Carcinoma	8
J K	48	M	2.5	Mucoepidermoid Carcinoma	21
J W	64	F	2.5	Fibrosarcoma	13

demonstrated that the tumor was not resectable without such sacrifice. Accordingly a segment of the main trunk of the nerve and of its adjacent plexus was removed with the tumor and a portion of the parotid gland.

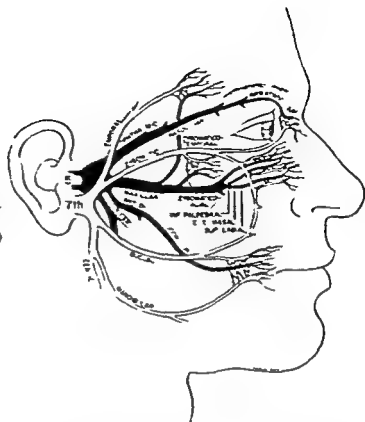
The lengths of the resected segments ranged from 2.5 to 5 cm. No attempt was made in any of the 10 cases to resuture or to graft. To relieve the more serious aspects of the obicularis oculi paralysis a fusion of the eyelid was routinely made on the affected side at the completion of the main operation. There was immediate and complete paralysis postoperatively of the corresponding facial musculature in all of the cases. In the 7 cases of spontaneous recovery the patients themselves were the first to discover that some active motion had returned. The time from operation to the first note of motion varied from 6 to 21 mo. as shown in the last column of Table 1.

The first evidence of motion in all patients had been the ability to pull back voluntarily the angle of the mouth. Once initiated there was a progressive and steady improvement in the degree and extent of recovery. Usually the muscles of the lower lip, cheek, eye, and forehead, in that order, began to show voluntary motion.

Once the voluntary motion returns to any part of the face it is as selective in that local area as in the normal opposite side. The droop of the angle of the mouth, so characteristic of 7th nerve paralysis, completely disappears. On forcible or exaggerated motion there usually remains some disparity between the 2 sides of the face, but in repose and in ordinary facial motion the difference is so slight as to be unobjectionable. We have reason to believe that such recovery of motion is favored and hastened if the patient actively and consistently (daily) practices in front of a mirror.

One of the deterrents and handicaps in the detection and study of this phenomenon is the natural disbelief of the surgeon who, having resected

Fig 2 Diagrammatic representation of the nerve anastomoses between the 5th (Trigeminal) and the 7th (Facial) cranial nerves



a complete segment of the facial nerve cannot bring himself to accept the patient's claim of voluntary motion. Another and even more difficult problem is presented when after a poorly conceived or unnecessarily traumatic operation is carried out a total 7th nerve paralysis ensues. After a few months or longer function begins to return and the surgeon unreasonably concludes that he merely overstretched or crushed the nerve at the operation while what may have actually happened was that he severed the main trunk and a similar phenomenon as herein described has caused the return of function.

In brief should there be functional disability of the 7th nerve (either partial or complete) after a parotid operation the actual physical status (section or lesser trauma) of the nerve cannot be known unless during the operation the main trunk and part of the plexus has been clearly exposed and identified and then either deliberately sacrificed or its continuity carefully preserved.

From the purely theoretical standpoint there are several possible explanations for the return of function following complete section with loss of substance of the 7th cranial nerve.

Spontaneous Regrowth of Motor Fibers Across a Defect of Several Centimeters. We feel that the unlikely chance regrowth of fibers across a defect at least 2.5 cm in width from a single trunk over to many distal stumps and also in view of the fact that we have observed no mass motion in the affected muscles render such an explanation untenable.

Decussation and/or Anastomosis Across the Midline of the Face. Since there is good selective motion of the involved muscles without motion of the contralateral muscles we believe this explanation also to be incorrect.

Establishment of New Motor Pathways Through the 5th Cranial Nerve.

(Trigeminal) According to anatomists the trigeminal nerve is divided into 3 divisions the ophthalmic and maxillary divisions thought to be purely sensory and the mandibular division which is both motor and sensory. In addition there are described (usually in fine print) a number of anastomoses between the terminal branches of the 5th and 7th nerve whose precise functions are unknown. These are shown diagrammatically in Figure 2. Practically every major branch of the 7th nerve is supplied by an anastomosis with the 5th nerve. What at first may seem a minor anatomical detail may actually have considerable significance. Such terminal communications must obviously be closely associated with the motor end plates and to us it seems reasonable that following complete and permanent interruption of the motor pathway, impulses may find their way from the cortex through the 5th nerve to the respective muscles. Although there may be some reluctance to accept this theory that new motor pathways may be established by way of the 5th nerve it seems to us to be the most reasonable explanation for the uncontestable fact that we have observed functional recovery in 7 cases despite segmental defects of several centimeters in the main trunk and plexus of the 7th cranial nerve.

SUMMARY

1 Of 150 consecutive cases of malignant neoplasm of the parotid gland treated in the years 1949 to 1958 inclusive 40 patients had a segment of the 7th cranial nerve (facial) varying in length from 2.5 to 5 cm. deliberately excised incident to their operation.

2 Of these 40 patients 28 lived free of disease for over one yr.

3 Of these 28 determinate patients 7 had a definite return of function after periods varying from 6 to 21 mo. without nerve grafts or any type of neurotomy.

4 We believe this return of function to be due to a growth of motor fibers through the 5th cranial nerve to the anastomosis between the 5th and 7th nerves and thus to the involved muscles.

THORACIC CORDOTOMY UNDER EPIDURAL BLOCK ANESTHESIA*

FREDERICK MURTAGH LEROY W. KRUMPHOLZ MARY RUTH WESTER

It has been well established that successful sustained relief of intractable pain by anterolateral cordotomy depends upon the establishment of adequate levels of complete analgesia at the time of the operation. For this purpose local infiltration anesthesia supplemented with pentothal and nitrous oxide or Trilene has been advocated. The patient is asleep during

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the initial phases of exposing the cord. He is awakened at the time cuts are to be made into the cord. Sensory testing is performed after the spinal thalamic tracts are sectioned and the areas of analgesia thereby produced are determined. If necessary supplementary cuts are made until the desired levels of complete analgesia are obtained. The patient is put to sleep again for the closure.

Numerous operators have found this method unsatisfactory. Thoracic laminectomy is a formidable procedure to perform under local infiltration anesthesia alone and often moderate to large amounts of supplementary anesthesia are needed to make the patient comfortable during the preliminary phases of the operation. Under these circumstances it is often difficult to arouse the patient to the full levels of consciousness necessary to make subjective responses to sensory testing reliable. In a state of partial dulling of the sensorium the difference between complete analgesia and a high grade hypalgesia may not be well appreciated by the patient. An incorrect interpretation of sensory loss may result. In addition under local infiltration and with the patient awake manipulation of the sensory nerve roots is painful and distracting to the patient. This pain may be so severe that the subjective response to sensory testing is further impaired. The surgeon in his natural desire not to cause undue pain for the patient is restricted in his ability to manipulate the cord for proper cutting and he may technically defeat himself. The advocates of the use of general anesthesia for cordotomy prefer the anatomic accuracy possible with the patient asleep.

In order to provide total surgical analgesia at the operative site and still have the patient awake for sensory testing we have employed epidural block analgesia.

Epidural analgesia is a segmental analgesia produced by the deposition of local anesthetic solution into the epidural space.

The exact site of action of the epidural analgesics is not clear but 3 possibilities exist. 1 diffusion occurs across the dura into the subarachnoid space and cerebrospinal fluid effecting a true spinal analgesia. 2 the drugs act directly on dural covered nerve roots in the epidural space. 3 the nerves are affected distal to the dural sheaths after they have left the intervertebral foramina producing a paravertebral block.

METHOD

Having received preoperative medication the patient is placed in the lateral decubitus position with the knees and neck flexed as far as possible. The skin over the back is aseptically prepared. A skin wheal is raised in the interspace between T 7 and T 8 or T 8 and T 9. A #16 gauge Touhy Huber needle is introduced through the skin wheal and directed cephalad. The degree of cephalad direction will be determined by the anatomic considerations of the interspace chosen. As the needle is introduced considerable resistance is commonly met at 3 points where ligamentous structures are encountered. The first of these is at the supraspinatous ligament. Definite but less marked resistance is encountered when the needle reaches the infraspinalous ligament. The third point of principle resistance is encountered when the ligamentum flavum is reached. After the needle has reached the ligamentum flavum the stylet is withdrawn and a drop of sterile procaine or saline is placed into the hub of the needle. The needle is then

advanced carefully thru the ligamentum flavum until a definite snap is felt indicating penetration of the ligament and entry into the epidural space. At this point the solution in the hub of the needle disappears into the shaft of the needle (the so-called drop sign of Gutierrez).

A #3 Fr polyvinyl catheter is now introduced thru the needle and directed cephalad, and advanced 3 to 4 cm. After the catheter has been advanced sufficiently the needle is withdrawn over the catheter leaving it in the epidural space. The catheter is now secured in place with adhesive tape in such a manner as not to be in the operative field.

An infusion of 5 per cent dextrose in distilled water is started and an initial reading of blood pressure is taken. At about the same time 2 cc of anesthetic solution is run into the epidural space. This is a test dose and serves 2 purposes: (1) if the catheter has inadvertently entered the subarachnoid space spinal analgesia will result; (2) if the catheter is in the epidural space a narrow band of analgesia will be produced corresponding to the level of the tip of the catheter.

After the test dose has shown the location of the catheter the initial epidural injection is made. The volume of anesthetic solution used depends upon the number of segments to be blocked and the location of the tip of the catheter.

In our experience if the catheter has been introduced as we have described an initial injection of 10 cc will produce analgesia extending from C-5 to T-9-10. Surgical analgesia will be complete in from 10 to 15 min.

A variable fall in blood pressure can be anticipated—therefore it is advisable to administer a prophylactic vasopressor prior to the injection of the anesthetic solution into the epidural space. The dose of vasopressor is determined by: (1) the anticipated number of segments to be blocked; (2) the patient's normal and preoperative blood pressures. If the prophylactic vasopressor proves to be inadequate an intravenous drip of dilute neosynephrine (0.02 per cent sol.) will be most effective.

The local anesthetic agents which may be used in this technique are: (1) xylocaine (Lidocaine) 1.5 per cent with epinephrine 1:200,000; (2) cyclaine 1.5 per cent (Hexyclaine); and (3) 1.5 per cent pontocaine hydrochloride (tetracaine). We prefer xylocaine or cyclaine because the onset of analgesia is rapid (6 to 12 min. as compared to pontocaine 15 to 30 min.). The duration of analgesia with xylocaine or cyclaine is from 45 to 75 min. In some cases it may even be longer. In the majority of cases the operative procedure will require from 1½ to 2½ hr., therefore we have injected a second dose of anesthetic solution just prior to the laminectomy. By following this procedure we have always had complete analgesia for the duration of the operative procedure.

All cases but one have been operated in the lateral recumbent position. This has been found to be most convenient since it is the position in which the anesthesia was given and is most convenient for the anesthesiologist to attend and question the patient during the sensory testing. If the procedure is a unilateral cordotomy the side to be cut is placed upward. No inconvenience has been encountered in doing bilateral cordotomy in this position.

Complete laminectomy is performed to provide adequate exposure of the cord. For unilateral cordotomy the laminae of T-2 and 3 are removed and the dura is opened in an elliptical fashion to the side of the intended cord

section. For bilateral cordotomy T 2, 3 and 4 are removed. The dura is opened in the midline and cut transversely on one side at the cephalic end and transversely on the other side at the caudal end of the exposure. The dentate ligaments are cut on both sides from their attachment to the dura. The ligament is then grasped with a long, straight forcep and the cord rotated medially to expose the anterior lateral segment. The arachnoid is opened and an avascular area on the cord is chosen. The cut is made with an unguarded bistoury. The knife is inserted at the dentate ligament and directed toward the center of the cord. It is advanced to a depth of 3 to 5 mm and then swept anteriorly to exit just in front of the anterior rootlets. Sensory testing is then performed. If supplementary cuts are necessary they are made and the patient retested until satisfactory levels of complete analgesia are obtained.

Epidural segmental block has been employed as the method of surgical anesthesia for 9 thoracic cordotomies. Five of these were bilateral procedures, 2 were unilateral and in 1 case bilateral section of the spinothalamic tracts was carried out in 2 stages. All have been for relief of intractable pain in the low back, perineum or lower extremities secondary to either carcinoma or trauma.

The operation was well tolerated by all patients including two who were poor operative risks because of associated cardiovascular renal disease.

All of the patients were sufficiently awake and alert for reliable responses to sensory testing during the sectioning of the cord. One patient on whom the bilateral procedure was performed in 2 stages was not able to give completely accurate responses to sensory testing at any time because of a severe emotional disturbance. The operator, Dr. H. T. Wycis, was satisfied, however, at the time of the operation that complete analgesia below T 7 was produced by the cuts into the cord. This was substantiated by the repeated postoperative testing although the patient was not initially relieved of her original complaint of painful muscle spasm in the lower extremities.

None of the patients exhibited undue discomfort because of the surgical manipulation. Stimulation and maneuvering of the sensory roots caused no discomfort. The cord could be easily mobilized in each case and accurate anatomical section of the spinothalamic tracts could be performed with assurance. In spite of this in only 3 of the 14 individual anterolateral cord sections was a single cut sufficient to produce complete analgesia at a satisfactory level. In the remaining 11 sections one or more supplementary cuts were necessary. For the most part the supplementary cuts had to be made posteriorly toward the dentate ligament to produce analgesia in the last 3 or 4 sacral segments.

In the majority of cases the area of segmental analgesia produced by the epidural block extended from C 7 or T 1 to T 8 or 9. In 1 case it ascended to C 4 producing respiratory embarrassment. In 2 cases it descended to T 12 and L 2 respectively making it impossible to accurately determine the upper levels of analgesia produced.

Seven patients obtained permanent relief of pain although in 1 of these cases there was a postoperative drop of 5 dermatomes in the sensory level on each side. It is worthy of note that 1 patient who had in addition to pelvic and generalized leg pain, severe root pain in the fifth lumbar root

voluntarily informed us on the operating table that she could feel the sudden relief of the root pain at the moment the cord was cut. She described the sensation as one of sudden exacerbation of the pain followed by immediate relief of all pain. This patient maintained complete relief of all spontaneous pain, but on the second postoperative day developed hyperalgesia and dysesthesia to touch in the fifth lumbar dermatome.

A second patient voluntarily described sudden electric like pain in the corresponding leg at the moment the spinothalamic tract was sectioned on each side.

CONCLUSIONS

Advantages Epidural block analgesia is superior to general anesthesia in thoracic cordotomy because (1) it avoids the cumbersome technique of endotracheal anesthesia and (2) it produces complete surgical analgesia and yet allows the patient to be fully awake and responsive for sensory testing at the time cuts are made into the cord.

Epidural block analgesia is superior to local infiltration anesthesia because (1) much smaller quantities of supplementary anesthetic agents are necessary (2) the operator is afforded complete freedom of movement and manipulation of the cord and nerve roots without causing distracting pain to the patient.

Disadvantages Epidural analgesia requires the skill of a trained anesthesiologist. In the hands of an inexperienced operator the failure rate may be high due to an inadvertent subarachnoid injection or inability to identify the epidural space.

With this method analgesia is produced in the thoracic segments which may extend as low as T 12 or L 2. It may be impossible therefore if one is seeking a high level of analgesia by cordotomy to determine the upper levels at the operation. However since this procedure is more often performed for pelvic and lower extremity pain this disadvantage may not be a serious deterrent to its use. On the other hand if a high level is desired the anesthesiologist can make an effort to keep his lowest level of analgesia as high as possible.

Complications Moderate to large falls in blood pressure may occur if too large a dose is used or if the proper prophylactic measures are not taken. There may be systemic reactions to the local anesthetic agent if large doses are required to obtain the desired spread of analgesia. These 2 disadvantages may be avoided by the proper placement of the catheter. Respiratory distress may be present if the concentration of the drugs used is greater than described or if the upper level of analgesia extends upward to include C 3-4-5 resulting in paralysis of the diaphragm. If a poor aseptic technique is used there could be contamination of the epidural space, the meninges and the epidural space.

Contraindications This technique is contraindicated if there is sepsis of the skin of the back at the site of the needle puncture and if there is known pathology in the epidural space or involved vertebrae. It is unwise to introduce a catheter into the epidural space if the prothrombin time is below 35 per cent of normal.

THE RECORDING OF ROENTGEN IMAGES OF SMALL BONE SPECIMENS USING XERORADIOGRAPHY*

EICHIRO ASAI CRAWFORD J CAMPBELL AND JOHN I ROACH

The recording of roentgen images of very small bones or fragments of bone from embryos or immature animals has been difficult.

Roach¹ in 1953 showed the superiority of xeroradiography in the study of wire screening of known size. In this method an electrostatic image produced on the surface of a semiconductor such as selenium is made visible by utilizing negatively charged powder granules.

Campbell, Roach and Grisolia showed how this resolving power of xero plates could be utilized to demonstrate bone detail in thin sections of normal and abnormal bone specimens. They confirmed the finding of Oliphant² and Hills and Stanford³ that in a xeroradiograph there is a slight exaggeration of detail due to an increase of powder to one side of a charged area and a corresponding decrease of powder immediately adjacent to it.

The purpose of this investigation is to test the use of xeroradiography in the demonstration of bone detail in rat and human embryos.

METHOD

Regular and two-fold enlargement xeroradiographs and conventional roentgenograms were taken of the following: 1 Rat embryos removed at weekly intervals from conception until maturity. 2 Human embryos of approximately 4 mo of gestation. 3 Individual embryonic bones (human).

Enlargement radiographs were obtained by placing a small focus (0.3 mm) tube 17" above the specimen and the plate exactly the same distance below. The original xeroradiographic images were recorded photographically using 35 mm Type A Kodachrome film and were enlarged by the photographic method to the same size as the conventional roentgenograms.

RESULTS

In the study of the rat embryos it is noted that at 10 days gestation there is no bone detail but soft tissue detail stands out more clearly in the xeroradiograph (Fig 1 A, 1 B). Bone detail can be noted in the rib and skull in the xeroradiographs of the 2 wk old embryo (Fig 2 B) whereas a routine roentgenogram of the same specimen (Fig 2 A) reveals only soft tissue. With further maturity the bone detail is always more pronounced in the xeroradiograph than in the roentgenogram (Fig 3 A, 3 B).

Soft tissue detail is sometimes quite pronounced in the xeroradiograph as contrasted to the routine roentgenogram. This is well illustrated in the hemi-

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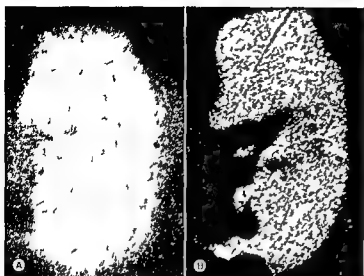


Fig. 1 A Rat Embryo 10 days gestation Roentgenogram (50 MAS — 55 kV) enlarged 10 times by photographic technique

Fig. 1 B Xeroradiograph (50 MAS — 50 kV) enlarged 10 times by photographic technique

Fig. 2 A Rat Embryo 16 days gestation Roentgenogram (50 MAS — 55 kV) enlarged 7 times by photographic technique

Fig. 2 B Xeroradiograph (50 MAS — 50 kV) enlarged 7 times by photographic technique



Fig. 3 A Rat Embryo new born Roentgenogram (50 MAS — 55 kV) enlarged 5 times by photographic technique

Fig. 3 B Xeroradiograph (50 MAS — 50 kV) enlarged 5 times by photographic technique

Fig 4 A Human Embryo approximately 4 mo gestation Roentgenogram (50 MAS - 53 KV) reduced in size by photographic method to 1/2 actual size

Fig 4 B Xeroradiograph (40 MAS - 60 KV) reduced in size by photographic method to 1/2 actual size

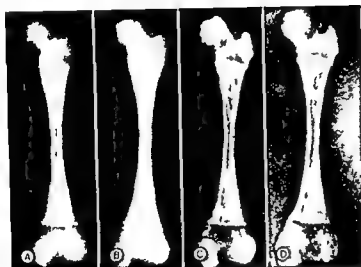


Fig 5 A Femur of human embryo approximately 4 mo gestation Roentgenogram (50 MAS - 53 KV) enlarged by photographic methods 2X

Fig 5 B Roentgenogram (50 MAS - 53 KV) enlarged 2X by radiographic enlargement technique

Fig 5 C Xeroradiograph (40 MAS - 60 KV) enlarged by photographic methods 2X

Fig 5 D Xeroradiograph (40 MAS - 60 KV) enlarged 2X by radiographic enlargement technique

section of the human embryo of 4 mo gestation in the demonstration of the ventricles of the brain, tongue larynx trachea and esophagus (Fig 4 A 4 B)

In the study of routine roentgenograms of embryonic bones better detail was obtained by enlarging the routine roentgenogram by photographic (Fig 5 A) rather than by radiographic enlargement technique (Fig 5 B) This may be accounted for on the basis of difficulty in getting good contrast in xeroradiography however the radiographic enlargement technique gave better detail (Fig 5 C 5 D)

DISCUSSION

In this study of embryonic bone specimens the definition and contrast gradation in both bone and soft tissue in the xeroradiograph are more pronounced than in the conventional roentgenogram. In the area of decreased deposit the fine detail may be diminished or obliterated. This was called the pull in effect by Oliphant.²

Artifacts in the form of small spots were often present. They were frequent when the humidity in the atmosphere was high and on the older and more defective xeroplates. Such artifacts although they obscured some detail were not easily confused with anatomic structure. The humidity may affect the size of the granules thereby altering the clarity of the image.

The image obtained in both bone and soft tissue detail in enlargement xeroradiography shows a contrast gradation superior to that obtained in conventional enlargement radiography.

CONCLUSIONS

Xeroradiography and enlargement xeroradiography are useful aids in the roentgenographic study of small bone specimens.

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THE HISTOGENESIS OF THE FUSION PROCESS AND THE USE OF TRANSVERSO FACET BONE BLOCKS IN SCOLIOSIS

J VERNON LUCK

It has been said that a successful research project ultimately poses more problems than it solves. By this definition this research project involving scoliosis has already been successful.

At the outset it was our hope to establish the manner in which the fusion segment grew in size following spinal fusions during childhood. The study was soon expanded in an effort to understand the causes of stress pseudarthroses. The incidence of pseudarthrosis following spinal fusions is distressingly high and it is natural that a histologic study of the fusion process would run head-on into the problem of the pseudarthrosis. The pseudarthrosis aspect of the investigation has included a study of mechanical stresses that are brought to bear on the fusion segment particularly bending and torsion stresses. This led us to use the transverso facet bone block a technique designed to create a compression arthrodesis particularly at the apex

of the primary curve where bending stresses are liable to develop to a high degree and thereby create a pseudarthrosis.

Histogenesis of the Fusion Process and Mechanism of Growth of the Fusion Segment When events proceed through a normal sequence the steps by which a fusion is achieved are similar to the stages in the healing of a fracture. Most of the callus that forms is bony in type although we have seen several instances of cartilaginous callus formed in a varying degree. Fibrous bone forms abundantly and bridges the fusion segment. Gradually the fibrous bone is replaced by mature lamellar bone completing the fusion process.

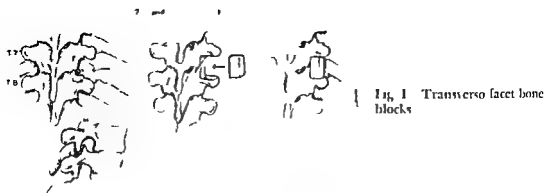
There has been an interesting polemic through the years centered around whether or not the fused segment of the spine underwent growth. Since there is no phenomenon of intrinsic growth in bone such as we see in cartilage it was reasoned that there could be no growth of the fusion block. Obviously there are no enchondral lines to increase growth by enchondral ossification. Theories to the contrary notwithstanding definite growth in the fusion block has been identified in numerous instances by Risser, Hallock and others.¹ The mechanism of this growth has not been clearly established.

METHOD

In the experimental phase of this study spinal fusions were carried out on white rats which were approximately one third grown. A segment of Kirschner wire has been inserted to lay on the dorsal surface of the laminae extending above and below the fusion segment. This was used for immobilization. A second Kirschner wire was placed in the same location except that this second wire was cut the exact length of the fusion segment. This gave an accurate index of the original length of the fusion.

The study thus far has been a basis for two observations: (1) The fusion block grows in size during growth. (2) The mechanism of growth appears to be one of simple periosteal ossification. This periosteal ossification appears in two forms: (1) A layer of osteoblasts on the surfaces of the fusion block creates layer upon layer of periosteal new bone. These layers are rather promptly assimilated and soon appear as lamellar bone. (2) The second type of periosteal ossification is in the form of trabeculated periosteal fibrous bone. This is particularly evident earlier in the fusion. This fibrous bone forming on the surface of the fusion block and at each end is steadily replaced by mature lamellar bone. In the past we have underestimated periosteal ossification as a mechanism of growth. It is our present belief that this simple mechanism is the explanation of growth of the fusion block during childhood.

Stress Pseudarthroses No attempt will be made here to present a detailed discussion of pseudarthrosis in spinal fusions. Our study of this aspect of scoliosis is far from completed. The study thus far relates to what we term the stress pseudarthrosis. The most common type appears to be this stress type and it generally appears at the apex of the curve where substantial corrective forces have been exerted. A damaging bending force can appear in the fusion block in any months after the fusion procedure. If the cast does not securely hold the correction achieved in the curve then bending forces frequently strong and inexorable try to recreate the original primary curve. It is necessary to securely hold the curve in correction following the fusion.



until the fibrous bone created early by the fusion process is replaced by lamellar bone. Even with mature lamellar bone an osteolytic process can develop at the apex of the curve when the load of bending stress is extreme and the size of the fusion block is thin and narrow.

In general the greater the prefusion mobility the greater the incidence of pseudarthrosis but equally and probably even more important the greater the force required to correct the primary curve the higher the incidence. Older age groups have a higher incidence.

In general the longer the fusion area the higher the incidence of stress pseudarthrosis. It is therefore in good order to search for methods and techniques that promise a reduction in the incidence of pseudarthrosis and this led us to utilize transverso facet bone blocks.

Transverso Facet Bone Blocks It is now well established that compression forces are favorable and are capable of stimulating the fusion process. Like wise bending and torsion forces are capable of retarding and thwarting the fusion process. In studying the anatomy of the spine the close relationship between the facet joints and the transverse processes becomes evident. In the dorsal spine the transverse processes are readily visualized and a bone block can be easily inserted between two transverse processes and against the denuded facet joints. In the technique of preparing a bed and inserting the bone block the facet joint is clearly visualized as is also the base of the transverse process above and below the facet joint. Bone blocks are inserted

Fig. 2 Transverso facet bone blocks at the apex of a scoliotic curve on the concave side. Bone blocks are used in conjunction with a Hibbs fusion.

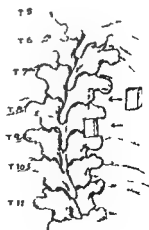
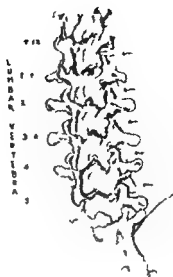


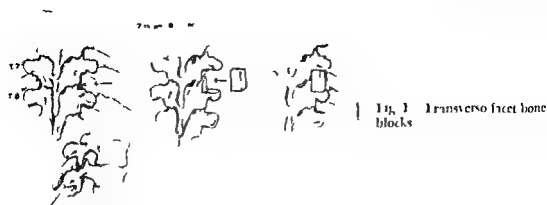
Fig 3 Transverso-facet bone blocks in the lumbar region



on the concave side of the curve and in most instances have been limited to the apex of the primary curve. At the base of the transverse processes a squaring is carried out and the margin of the facet joint excised. With the squaring of the base of the transverse process above and below and the excision of the margin of the facet joint a bed is created to receive a rectangular block. A bone block is then prepared and is taken from whatever source is desired. Bone blocks have been prepared from a variety of sources which include the tibia, the ilium, the spinous processes, the ribs, and a segment from a fusion block created by a previous operation. From 1 to 3 blocks are used and these are inserted at levels at and adjacent to the apex of the primary curve on the concave side. The transverse processes are spread although not much spreading can be achieved as a rule. The bone blocks are inserted by tapping them into place giving them a tight fit. With increase in the bending stresses which so often happens postoperatively compression forces are increased at the most vulnerable site of the fusion segment which is the apex of the curve. A second use has been made of the bone blocks by utilizing them in pseudarthrosis following conventional fusions. After excision of the pseudarthrosis and the creation of multiple bone chips across the site of the pseudarthrosis cleft a bone block has been inserted between the two transverse processes and against the facet joint directly at the site of the pseudarthrosis. The results from this technique are most encouraging but further observation will be required and long range results studied before final conclusions can be drawn. Transverso-facet bone blocks have been used in nine cases. Three of these were pseudarthroses and six were in conjunction with Hibbs type spinal fusions.

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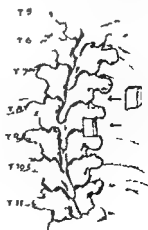
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Fig. 8. Transverso facet bone blocks at the apex of a scoliotic curve on the convex side. Bone blocks are used in conjunction with a Hibbs fusion.



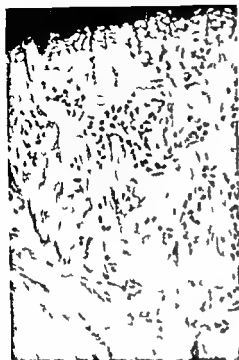


Fig. 1 Photomicrograph $\times 300$ showing 70 day old rat after 49 days on a diet extremely low in calcium and vitamin D. Epiphyseal cartilage appears across the top of the picture. Mast cells appear in the endosteal connective tissue throughout cancellous bone. Periosteum and muscle shown in the lower left side of the picture contain few or no mast cells. Hematoxylin eosin and azure II stain.

growth 4 to 6 wks later or at about 12 wks of age. Mast cells appeared precipitously in the experimental animals at about 4 wks on the diet and rapidly increased in numbers until the bone was saturated with as many as 200 per high power field ($\times 450$).

Fracture healing (the formation of new bone tissue) occurred in these animals about as rapidly as in normal animals. Callus formation was exuberant. The fracture site seemed to hold the highest priority in the body for the building stones necessary for osteogenesis. Calcification of the new bone and fibrocartilaginous callus was slightly delayed however and as in the metaphysis of the same bone there was a certain amount of osteoid and chondro-osteoid. There were no mast cells in the callus during the first 3 wks of healing when there were proliferating osteoblasts and while new bone formation was rapidly in progress. After 3 wks of healing when osteogenesis was slowed down and marrow appeared in the callus mast cells began to appear first in small numbers outside the endosteum and then at greater numbers in place of the endosteum.

Fifteen littermates reared on the experimental diets were treated with large doses of vitamin D (Calciferol 900 000 I U per cc daily for 7 days). The rachitic metaphysis was absorbed and mast cells were degranulated and disappeared into the bone marrow.

Fifteen experimental animals were also treated with large doses of parathyroid extract (1 000 I U per day for a period of 7 days). Parathyroid hormone superimposed more extreme lesions of osteitis fibrosa upon the complex pathological picture of the bones caused by the calcium deficiency. Osteitis fibrosa appeared to favor the formation of mast cells.

DISCUSSION

The function of the mast cell is not known. The following substances have been claimed to form in the granules of mast cells: histamine, heparin,

MAST CELLS IN BONES*

MARSHALL R. URIST AND FRANKLIN C. MCLEAN

Striking formations of mast cells were encountered by chance in calcium deficient animals in the course of studies upon bone formation and repair.^{1,2} Mast cells are normally located in the bone marrow and rarely ever near bone tissue. In rats fed a diet extremely low in calcium and vitamin D (Shohl's Diet E)³ mast cells increased in numbers in the endosteum from 0 to 5 to 150 to 200 per high power field. These observations were possible because of the authors' routine use of hematoxylin-eosin and azure II staining of undecalcified sections of all of their experimental material. Hematoxylin and eosin, the stains generally used for histological work on bone, scarcely reveal the presence of mast cells. The work presented in this paper began in 1937 and is submitted to this Forum because of the great interest in mast cells currently in all fields of biology and medicine.

The bone disorder produced in rats fed Shohl's Diet E is a combination of moderate rickets (secondary to the vitamin D deficiency), osteoporosis secondary to the calcium deficiency, and osteitis fibrosa resulting from stimulation of the parathyroid glands⁴ (possibly by the low level of calcium in the serum). The rickets was evident by osteoid tissue around the bone trabeculae, the osteoporosis by the low volume of bone and the attenuation of the trabeculae, the osteitis fibrosa by the increased numbers of osteoclasts and the large amount of fibrous tissue between the trabeculae.

The new location and the appearance of increased numbers of mast cells was not accompanied by an increase in the mast cell population in the mesentery of the same animals or the synovial membranes of the joints of the same bones in the same individual. It is characteristic of the mast cell to find it in clusters in various locations in the body, as for example: (1) a membrane such as the pleura of mesentery, (2) a perivascular connective tissue focus such as in skeletal muscle, heart or visceral organs, (3) a complex tissue such as bone marrow, (4) a pathological tissue such as a clinical or experimental tumor. The endosteal location resembled the perivascular connective tissue location but it differed in that it had a distinct pattern of its own. The mast cells were on the surface of, within, and even beneath the connective tissue cells lining the bone trabeculae. There were however numerous mast cells surrounding small and large blood vessels adjacent to bone tissue. In young growing animals osteoblasts are normally found in this location.

METHOD

Mast cells accumulated in the bones in calcium deficient rats when growth was arrested between 3 and 4 wks after the animals had been fed the experimental diet. Rats reared on control diets reached the plateau of their rate of

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seventh decades. Hence despite the absence of quantitative experimental evidence it seems apparent that the difference in velocity of healing is greater between infant and adult in fracture healing than in soft tissue healing. It also seems apparent that fracture healing velocity may reach a plateau in late adolescence and early adult life with little if any subsequent diminution in velocity.

If these opinions are true we must look elsewhere than to the chronological age of bone alone as an explanation. The epiphyseal cartilage has a life history which parallels the increased velocity of fracture healing in the growing animal. It is at least logical therefore to suspect that the governing factor regulating fracture healing velocity may either reside in the epiphyseal cartilage or accompany its presence. The occurrence of pathological fractures in the long bones of weanling rats is differentiated from adult animals when exposed to massive doses of Vitamin A is well described and discussed by Wolbach.³ This fact is additional evidence that the epiphyseal cartilage is not only an anatomic unit contributing to bone growth in length but may be a physiologic unit upon which other aspects of bone physiology depend.

METHOD

To investigate this fractured femora in growing and late adolescent guinea pigs were examined utilizing Vitamin A in excess as a tool to unblock normal epiphyseal physiology.^{3,4}

Vitamin A in oil in a 750 000 international unit per cc concentration was administered 3 times weekly in a dosage to be equivalent in total to 500 international units/gm/day. The drug was not begun until 1 wk after fracture and the oil preparation and intermittent dosage were utilized to limit

Fig. 1 Cross appearance of section through untreated infant femoral shaft fracture. The callus nidus is apparent even at this minimal magnification and is cartilaginous.



hyaluronic acid or some other acid mucopolysaccharide precursor of ground substances acid phosphatase alkaline phosphatase phospholipid, lipids, lipase peroxidase, cytochrome oxidase unidentified proteins free iron Of these substances alkaline phosphatase and ground substance precursors are 2 that could be stored in mast cells when bone formation ceases in calcium deficient animals

The mast cell requires further study in animals and humans with various disorders of bone such as rickets osteomalacia osteoporosis and osteitis fibrosa Its role could be to store alkaline phosphatase but it is actually as much of a riddle in bone as in all of the other connective tissues of the body⁴

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THE INFLUENCE OF VITAMIN A INDUCED EPIPHYSEAL CARTILAGE ABNORMALITIES ON FRACTURE HEALING IN THE GUINEA PIG*

EDWIN G BOVILL JR

The velocity of the healing process in skin and in fibrous connective tissue can be expressed quantitatively and undergoes a steady decline with age^{1,2} The velocity of healing in a soft tissue wound in the young animal is only a fraction faster than in the adult The young adult will heal a soft tissue wound measurably faster than the older adult I agree with Harvey² that healing of a soft tissue wound appears to involve reactivation of a normal growth process

A difference seems apparent on comparing fractures with soft tissue wounds from the point of view of velocity of healing Quantitative methods of measurement of fracture healing are not available Clinical information in the human substantiates the opinion that long bone fractures in the first decade of life heal at least twice as fast as comparable fractures in the third and fourth decades and possibly three times as fast Furthermore very little if any comparable difference in velocity of healing is present when comparing fractures in the third and fourth decades with those in the fifth sixth or

*From the Department of Surgical Research Stanford Medical School San Francisco Calif Aided by a grant from the California Division of the American Cancer Society

guinea pigs ($225 \text{ gm} \pm 10$) were utilized. Digital fracture of the right femoral shaft was carried out on all animals and they were then separated by random selection into 4 groups of 10 each. Ten of each age group received no treatment. The other 10 of each age group received Vitamin A in oil as outlined above. At 5 wks. all animals were sacrificed. Microscopic sections were prepared in the longitudinal axis in either sagittal or coronal plane whichever best permitted the section to pass through the center of the fracture and through the distal femoral epiphysis.

Seven of the 40 animals were lost to the end result either through death early in the experiment or by presenting hip dislocation, epiphyseal separation or no fracture at the time of sacrifice and examination. The one distinction sought was the presence of either cartilage or fibrous connective tissue at the fracture callus nidus.

Fracture Callus Nidus Infants—

Treatment	Cartilage	Fibrous	Conn. Tiss.
Control	7	0	7
Vitamin A in Excess	1	6	10
	11	6	17

Fracture Callus Nidus Adolescents—

Treatment	Cartilage	Fibrous	Conn. Tiss.
Control	7	0	7
Vitamin A in Excess	7	1	8
	14	1	15

The treated 3 wk. old animals and the untreated and treated adolescent animals did not change weight appreciably over the period studied. The untreated 3 wk. old animals gained to an average weight of $100 \text{ gm} \pm 50$.

A similar experiment using tibial fractures was characterized by the absence of a cartilaginous bar in the callus in any of the animals. This appeared to be the result of close apposition of the fragments in the tibial fractures as compared with the rather wide separation typical of the femoral fractures. The tibial fractures in general demonstrated early trabecular bony bridging of the fracture at 5 wks. No definitive factor was found in the tibial fractures to permit comparison of the treated and untreated animals in a quantitative fashion.

SUMMARY

Previous experimental work with excessive dosage of Vitamin A has demonstrated that some of its action on bone may be the result of Vitamin A induced epiphyseal cartilage changes rather than direct.¹ The results in the group of femoral shaft fractures reported here agree with this concept.

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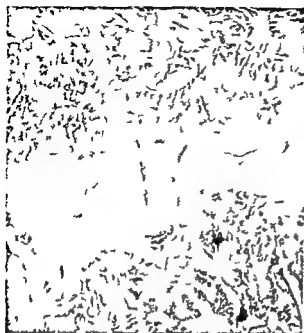


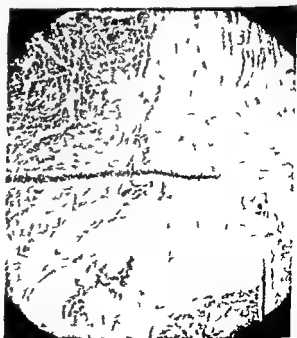
Fig. 2 Section through center of callus nidus in treated late adolescent Cartilage

the amount of handling to a minimum. That the vitamin was active was evidenced by the epiphyseal plate response which was always present although not of uniform degree. The lack of uniform response may represent uneven absorption from the depots of injection.

The lack of a definable end point to fracture healing prevented measurement of velocity of healing. At the 5 wk post fracture period all untreated animals exhibited a bar of cartilage between the fragments in the center of the callus. This was not uniformly true with the young treated animals as the central portion of the callus was frequently fibrous connective tissue. Accordingly the following experimental design was used.

Twenty late adolescent guinea pigs ($550 \text{ gm} \pm 50$) and 20 three week old

Fig. 3 Section through center of callus nidus in treated infant. Fibrous connective tissue



RESULTS

Observation of the injected joints of the sensitized animals during the first few hours revealed progressive swelling of both joints causing the animals to limp badly. Measurements of the amount of swelling were not carried out. There was less swelling of the joints of the non sensitized animals these animals likewise limped. Freund⁴ had previously shown that the paraffin oil in itself is a tissue irritant.

Cross examination of the joints in the sensitized group after sacrifice revealed marked engorgement of the blood vessels about the knees more marked in those containing the adjuvants. There was gross swelling of all the joints but most in the adjuvant containing joints. There was injection of the blood vessels of the controls also which was the most marked feature of the reaction of these joints.

Examination of the joint cavities of the sensitized group revealed a great increase in the amount of synovial fluid. In contrast the joint cavities of the control animals were dry free of any synovial fluid with the adjuvant clinging to the synovium and difficult to wash or pull free. At 24 hr the synovial fluid in the sensitized animals was bloody. This did not occur in those sacrificed at later dates though the overall amount of synovial fluid was increased. At 21 hr in the sensitized group the synovium was markedly thickened beefy red in color and encroached on the articular cartilage and the patella (Fig 1). The adjuvant containing joints showed the more marked reaction.

The reaction noted in the knee joints of the sensitized animals decreased from a marked reaction at 3 days and gradually decreased through the 28th day (Figs 2 & 3). In the non sensitized control animals there was never any synovial reaction or increased fluid except where the dry adjuvant was adherent to the synovium. One control animal had a grossly infected joint but the synovium even in this joint did not compare with those previously sensitized (Fig 4).

Histological sections of the synovium of the animals in which an anaphylactic arthritis was produced showed a greatly thickened synovial lining with villous formation and a marked inflammatory reaction as evidenced by the tremendous number of inflammatory cells (Fig 5).



Fig 1 Severe synovial reactions in the knees of a sensitized rabbit 24 hr after injection of 0.5 cc of Freund's adjuvants in the knee on the left and 0.5 cc egg albumin into the knee on the right

THE PRODUCTION OF ANAPHYLACTIC ARTHRITIS WITH THE USE OF FREUND'S ADJUVANTS*

RICHARD T. ODELL AND J. ALBERT KEE†

Local anaphylaxis or an Arthus phenomenon was demonstrated by Arthus in 1903.¹ This phenomenon or local anaphylaxis is the inflammatory reaction occurring when an animal is immunized against a protein and is re-injected with the same antigen, i.e., the reaction which occurs when antibody and antigen meet in the tissues. Friedberger² is generally credited as the first to produce local anaphylaxis in the joints. Many investigators have been interested in producing as well as preventing or controlling anaphylactic arthritis in experimental animals.

Freund³ and his coworkers found when they were elaborating on some older experiments that killed tubercle bacilli in paraffin oil produced complement-fixation antibodies in high titer for at least 18 mo. after injection. Diverse antigens such as proteins, bacteria, viruses, rickettsiae, and simple chemical compounds combined with paraffin oil alone enhanced and prolonged antibody formation but had little effect on sensitization. The addition of killed mycobacterium strikingly promoted sensitization with questionable increase in antibody production.⁴ Thus Freund produced a water in paraffin oil emulsion to which could be added a variety of antigens and produce antibody formation and sensitization. The water in oil emulsion with killed mycobacterium frequently bears the name of Freund's adjuvants.

In this experiment we used the adjuvant containing killed mycobacterium butyricum and added a protein antigen, egg albumin. Furthermore, quite by accident, the adjuvants were injected into the knee joint of a previously sensitized rabbit and the animal received such a severe reaction that we decided to continue the use of the adjuvant in the production of anaphylactic arthritis.

METHOD

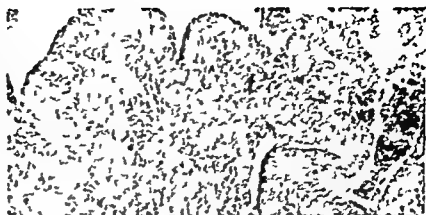
A group of young adult white rabbits of the same sex were selected and each received intramuscular injections of 2.0 cc. of Freund's adjuvants containing a 2.4 per cent solution of egg albumin. One cc. of the solution was injected into each of 2 sites deep in the muscles. At the end of 3 wks. skin tests with 0.1 cc. of the antigen injected subcutaneously gave each of the animals a typical Arthus reaction. The animals were then sensitized enough to have injections made into the knee joints. One half (0.5 cc.) of the egg albumin antigen was injected into the right knee and 0.5 cc. of the adjuvants injected into the left knee. A control group of animals which had not been sensitized received 0.5 cc. of the adjuvants into the knee joints.

The animals were observed for several hours on the day of injection of the joints and sacrificed with an overdose of ether at 24 hrs., 3, 5, 7, 10, 28, and 90 days respectively. Studies were made of the gross specimens which were then photographed and histologic sections made of the synovium, furs, pads, and any damaged articular cartilage.

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Fig 5 Typical inflammatory reaction and villous formation of the synovium following sensitization and injection of Freund's adjuvants into the knee joint of a rabbit



DISCUSSION

The production of anaphylactic arthritis or local anaphylaxis in laboratory animals has long been of interest to immunologists. The rediscovery by Freund that antigens incorporated in a water-in-oil emulsion and paraffin oil potentiated and prolonged antibody formation for long periods provided an excellent method for the local production of anaphylaxis. Local anaphylaxis or the Arthus phenomenon has been produced in the integument, the lungs, livers, and joints of animals. We had not seen well illustrated local or anaphylactic arthritis and by accident discovered the use of the Freund adjuvant technique as a means of producing this type of arthritis. We have succeeded in producing a severe anaphylactic arthritis in sensitized animals with Freund's adjuvants containing mycobacterium butyricum and egg albumin as an antigen. The adjuvants without the antigen produced a greater and more severe reaction than the antigen proper. Furthermore, a chronic arthritis was produced when the sensitized animal's knee was injected with the adjuvants and the animal allowed to live for 3 months or longer before it was sacrificed. The histological picture in the acute anaphylactic stage was one of acute inflammatory synovitis.

CONCLUSIONS

1. A different method of producing anaphylactic arthritis has been shown.
2. The use of Freund's adjuvants in the knee joints of previously sensitized animals consistently gave a severe local or anaphylactic arthritis.



Fig 6 This roentgenogram shows erosion of the articular cartilage of the bones of the right knee 90 days after the injection of adjuvants into a sensitized rabbit. The control left knee received no injection and shows no arthritis.



Fig 2 The reaction at 3 days On the right there is virtually no reaction to the egg albumin antigen The reaction to the adjuvants in the knee on the left is quite severe

Fig 3 The 7 day reaction in sensitized animals is subsiding but is still considerable



Fig 4 Control knees showing irritative power of the paraffin oil The reaction was greatest at 5 days

Several sensitized animals were kept for 3 mo after injection of their knee joints to see if a chronic arthritis could also be produced Figure 6 shows the arthritis produced in the right knee with the normal left knee as a control In the injected knee erosion of the articular cartilage of all the bones is seen and bony overgrowth is beginning to occur

extract of skin from 7 rabbits and received a graft from a donor not contributing to the pool. In the second experiments the donor rabbit provided the skin for both the extract and transplant. In the preparation of the pooled extract, as much full thickness skin as could be obtained was removed from the rabbits after clipping the hair and chemical depilation. The skin was minced after removal of all subcutaneous tissue and blood vessels and ground in a stainless steel ball mill at -70°C until the skin was converted to a fine powder. It was then extracted with buffered saline in a Waring blender at 1°C . The extract was sterilized by Seitz filtration. It contained 3 mg. of protein/ml.

Each rabbit received autogenous and homogenous transplants. A 2×8 cm full thickness area of skin was removed from both sides of the posterior thorax. The panniculus carnosus was left as the host bed. Each piece of skin was cut into eight 1 cm squares. Autografts were placed on the right side and homografts on the left. Exchange of homografts were effected by operating on pairs of rabbits. The graft areas were covered with vaseline gauze, a gauze holster tied over this and a light plaster bandage around the body. Either half or a whole graft was removed every 4 days for histological study.

Immunization consisted of 3 weekly subcutaneous injections of skin extract containing 25 mg of protein per injection with Ramon's adjuvant. Serum specimens were taken from recipient animals prior to the grafting operation and at weekly intervals thereafter. One group of rabbits was grafted at the start of immunization and another group was grafted 5 wks after the start of immunization. Three rabbits were used as controls with out immunization.

The individual extracts were prepared from large sheets of skin removed from the flanks of donor rabbits. The wounds were closed so that these rabbits could be used later as donors for skin grafts. In this 1 to 1 series all rabbits were grafted 5 wks after the start of immunization since this was the time of occurrence of peak titer in the bone immunization studies.

RESULTS

In the control rabbits the usual findings in autogenous and homogenous full thickness grafts were observed. The homografts turned dark brown to black and showed complete necrosis from mid dermis outward by 12 days. About 15 per cent of the full thickness autografts showed failure of take of the epidermal elements.

In 3 rabbits grafted at the start of injections of extract there was no difference in the fate of the homografts in treated and control animals.

Two rabbits were grafted 5 wks after the start of immunization with pooled extract and 9 rabbits with individual donor extracts. Since the gross and histologic results were similar they will be described together. Only those transplants which adhered to the host bed at the time of first biopsy are considered. At 4 days the gross and histologic appearance of the autograft and homograft were similar. At 8 days a light tan to purple color appeared in a few of the homografts whereas most of the autografts were pink. They both recovered color after blanching on pressure. Microscopically the usual autograft epidermal hyperplasia was evident while the homograft epidermal elements showed pyknosis of cells with infiltration of

3 These same adjuvants can be used to produce a chronic arthritis in previously sensitized animals

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THE FATE OF HOMOGENOUS SKIN TRANSPLANTS IN RABBITS IMMUNIZED WITH SKIN EXTRACTS*

M BONFIGLIO W S JETER AND W OSTRANDER

Attempts to obtain permanent survival of skin homografts in normal animals have met with uniform failure. It is well known that skin homografts incite an inflammatory reaction which leads to necrosis and slough of the graft at a predictable time. Efforts to modify the host reaction to skin homografts by injection of donor skin extracts have been reported recently. Allen and associates¹ reported prolongation of survival of skin homografts in rabbits by desensitization with saline extracts in which phenol was added as a preservative. Billingham and Medawar² however were unable to effect the survival time of skin homografts with phenol free donor skin extracts. On the other hand Hardin and Werder³ produced permanent survival in the majority of homologous skin transplants in heterologous CFW mice by subcutaneous injection of homologous skin extracts. They considered the results to be due to a process of immunoparalysis rather than desensitization. Previous studies on bone homografts by us⁴ have shown that preimmunization of recipient rabbits with extracts of donor rabbit bone appreciably reduce the inflammatory reaction to a subsequent frozen homogenous bone graft.

With these results in mind experiments were undertaken to see what effect injection of skin extracts would have on the fate of homogenous skin transplants in the rabbit.

Using skin as donor material the techniques previously developed for bone extracts were employed.⁴

METHOD

Large young male adult rabbits were used. Two series of experiments were performed. In the first series the rabbits were immunized with an

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THE EFFECT OF INTRAMEDULLARY NAILING ON THE GROWTH RATE OF IMMATURE CANINE HUMERS*

FREDERICK L. BEHLING AND JOSEPH M. JAMES

With the advent of the use of intramedullary fixation in the treatment of fractures many theoretical and practical problems have been introduced. One of these concerns the effect of metallic intramedullary fixation on the epiphyseal growth in an immature long bone. Blount¹ referred to the stimulation of the nail alone as being probably responsible for 1 to 2 cm. of overgrowth of the immature femur in cases of fracture treated by intramedullary fixation reported to him. Bisgard² in 1936 reported that fractures of the tibia in immature goats were found to produce more overgrowth in healing if internal fixation was used than if it was not used. He based this conclusion on results of increased overgrowth found in 2 cases in which a Lane plate was used for fixation.

By means of animal experiments we sought to evaluate the effect of metallic intramedullary fixation on the growth rate of an immature long bone, hoping to get a clearer conception of the role of the nail as an alleged irritant to the growing epiphysis. We selected the femur of the immature dog as a suitable bone. In each animal we operated on one femur leaving the other unoperated as a control. In one group of animals the femur was fractured and the fracture fixed by intramedullary fixation and in the other group the nail was placed the length of the intramedullary canal without producing a fracture.

MATERIAL

In this experiment 21 young dogs taken at random were used. In most the ages were not exactly known but were estimated to be between 4 and 6 mo. at the beginning of the experiment. The estimates of age were made by the veterinary staff of the Mayo Foundation.

The surgical procedures were performed with the animals under general anesthesia, aseptic technique being used throughout. The intramedullary fixation was accomplished by means of stainless steel Steinmann pins 3/32" in diameter, shaped roughly to a point at either end and inserted by means of a hand drill.

METHOD

In all animals one femur, alternately left and right, was operated upon, the other serving as a control. In one animal nonunion developed owing to a technical error and the intramedullary nail was extruded at the knee. In another animal a massive infection of the operated leg developed. These two animals are not included in the analysis of the experiment.

Group I consisted of 10 animals in which an intramedullary nail was inserted from proximal to distal without fracture of the femur. The superior aspect of the femoral neck was approached through a 1" incision and a 1/16" drill hole was made in the superior cortex of the femoral neck. The Steinmann pin was drilled down the medullary canal until it could be

*From The Mayo Clinic and Mayo Foundation, Rochester, Minnesota. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

inflammatory cells about these elements. By 12 days the homografts had become darker in color but not quite as leathery as the control homografts. A few grafts had proliferation of epidermis and hair follicles as well as infiltration of inflammatory cells. However, most of the homografts at this time showed sufficient necrosis and inflammatory exudate at the junction of the deepest epidermal elements and the dermis to indicate that this portion of the graft would slough. About two thirds of the dermis appeared viable. At 16 days the superficial necrosis of the homograft was certain. In a few homografts hyperplasia of the matrix cells of the hair follicles persisted as long as 16 days. From this time on there was gradual marginal invasion of the junction between necrotic and viable dermis by host epidermis until the eschar was completely loose at an average of 24 to 28 days.

In those instances where partial necrosis and slough of autografts occurred, autograft repair appeared identical to the repair of the homografts up to 28 days. The repair epidermis then developed projections into the dermis which appear like early hair follicles and sebaceous glands by 36 to 40 days. Indeed, homografts on 3 of the rabbits did grow fine hair. Apparently this arose from regenerating host epidermis which covered graft dermis, as recently shown by Breedis.⁵ The dermis of the homografts shrank gradually to about half the size of the autografts. As late as 134 days there was persistence of homograft dermal collagen in the immunized rabbit.

CONCLUSIONS

1. None of the epidermal elements of full thickness homograft survived transplantation beyond 16 days in rabbits injected with pooled skin extract or donor skin extract with adjuvants.

2. The homograft dermis in immunized rabbits persisted as long as 134 days.

3. Regenerating host epidermis which covered the homograft dermis developed hair and sebaceous glands.

4. Repair of partially necrotic full thickness autografts was similar to that of homografts.

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Fig. 3 Six weeks postoperative Group 1 femur showing growth about nail



were taken in this group also and follow up roentgenograms were taken in both groups to determine the progression of healing (Figs 3 and 4).

Following the surgical procedures the animals were returned to the kennels where they were given no special care other than 1 or 2 daily exercise periods. Considerable postoperative reaction was noted especially in the fractured femur group. It was noted that the operated legs were not used as weight bearing members in either group for about 1 wk after which all animals began to touch down on the operated leg. The gut was normal



Fig. 4 Two months postoperative Group 2 femur showing healing and growth about nail



Fig 1 Immediate postoperative Group 1 femur showing nail in place

palpated just emerging from the bone distally. It was then withdrawn to leave the pin just under the cortex distally. The pin was then cut off proximally as nearly flush with the cortex as possible and the incision was closed (Fig 1). Postoperative roentgenograms were taken to determine the position of the pin especially with respect to the distal femoral epiphysis.

In Group 2 consisting of 9 animals the femur was approached laterally and fractured in its mid third by means of a Gigli saw. The intramedullary nail was then inserted in the usual retrograde manner. After reduction of the fracture the nail was inserted to emerge at the distal end of the femur and then withdrawn as in Group 1 (Fig 2). Postoperative roentgenograms



Fig 2 Immediate postoperative Group 2 femur showing fracture and nail in place

Table 2 Intramedullary Nailing Group Averages

AVERAGE	FEMUR	
	FRACTURED	INTACT
Length of control femurs cm	14.5	16.3
Shortening after operation cm	0.47	0.35
Per cent of control	3.2	2.1

is crossed by the nail. In only 1 femurs as shown by the asterisks were the nails not across the epiphyseal line and thus may not be a large enough group to serve as a basis for judgment.

The important and statistically valid finding presented here is the fact that shortening occurred in all femurs with intramedullary fixation with or without fracture. Table 2 shows a comparison of the 2 groups with group averages for length of the control femurs, shortening of the operated leg, and shortening as a percentage of the length of the control femur. The shortening is greater in the fractured femurs as a group than in the intact femurs, and the degree of shortening expressed as percentage (line 3) also is greater.

COMMENT

Our purpose to assess the influence on growth of metallic intramedullary fixation in a growing bone is achieved in the finding of shortening in all but one of 19 immature canine femurs into which we introduced stainless steel Steinmann pins with and without fracture. Why the stimulation usually seen in humans and reported by Bisgard in animals following fractures of immature long bones was not seen by us is not known. Not only was there no growth stimulation in fractures but rather the series of fractured femurs was shorter as a group than the series in which the intramedullary nail was introduced without fracture. The fact that shortening was found in 18 of the 19 animals studied, including 10 femurs without fracture, disputes the role of the intramedullary fixation device as an irritative factor on the epiphysis.

CONCLUSIONS

On the basis of these experiments we conclude:

1. The presence of a metallic intramedullary nail in an immature canine femur does not produce an increase in length by virtue of its presence alone.
2. A slight shortening follows intramedullary nailing of immature canine femurs, slightly greater in bones which are fractured than in those without fracture.

Further investigation is needed to correlate these findings with comparable experiences in clinical cases.

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Table 1 Shortening of Femur After Intramedullary Nailing

NUMBER	TIME MONTHS	SHORTENING OF FEMUR AFTER OPERATION	
		Cm	PER CENT OF CONTROL
FEMUR NOT FRACTURED			
358	6	0.7	4.2
357	5	0.5	3.5
501	5	0.5	3.1
353	5	0.5	2.9
354	5	0.4	2.4
351	5	0.1	1.7
600	2	0.2	1.2
352	5	0.2	1.1*
498	5	0.1	0.6
603	5	0.0	—
FEMUR FRACTURED			
600	6	0.7	5.5
441	4	0.7	5.3
445	5	0.6	4.1*
442	5	0.5	3.5
602	6	0.4	3.2
491	5	0.4	2.9
601	5	0.4	2.5*
599	2	0.3	1.8*
491	5	0.2	1.2

*Nail not across epiphyseal line

and no evidence of protection was evident in Group 1 at 3 wks and in Group 2 by 6 wks.

The animals were killed from 2 to 6 mo after operation. All animals were killed at more than 10 mo and less than 1 yr of age at a time when epiphyseal growth had nearly been completed.

After death the hind legs were dissected free of soft tissue, sections were taken of the epiphyses and the bones were boiled to rid them of all soft tissue. Accurate caliper measurements were then made of the tibias and femurs in each animal. These measurements were repeated by a disinterested observer to minimize technical error. These measurements form the basis for analysis in this study. The tibias were not found to differ by a significant amount and no trend was noted in the differences that did exist. Study of the microscopic sections of the epiphyses showed no difference between the 2 groups. The results of femoral measurement showed a statistically significant difference between the 2 groups and are shown in Table 1.

Shortening occurred in all but one operated femur in the entire series and in that one the operated and the control bones were of the same length. The amount of shortening compared as percentage to the length of the control femur is shown in the right hand column. This column denotes the degree of shortening produced by the nail and the fracture and nail respectively.

The degree of shortening is not directly related to the time the nail was in place and seems to be unaffected by whether or not the epiphyseal line

Fig 1



union 1 dog died from a severe infection 2 dogs in whom the ligamentum teres was preserved had contrasting stripping procedures on the capsule 1 dog on whom the minor stripping was done healed in 34 days the other dog on which a major stripping was done developed aseptic necrosis (Fig 1)

Forty three animals had either omental or muscle (vastus gluteus rectus) grafts applied to the head or neck fragments immediately following fixation A $\frac{3}{16}$ drill hole was made in the distal fragments in an oblique direction as close to the fracture site as possible in some animals and subcapitally in the proximal fragments in others the various grafts were introduced into the drill tract and maintained by 1 or 2 silk sutures

Six animals had vastus lateralis muscle transplantation in 1 we used the gluteus minimus 5 had omental transplants to the middle of the neck area and 5 others had vastus lateralis grafts affixed to the sub capital area 3 had rectus abdominis grafts which included a branch of the inferior epigastric artery Ten other animals had omental grafts placed just behind the head Thirteen animals on whom various procedures were done died from a variety of causes

RESULTS

Our most difficult complication was a high infection rate This has been lowered considerably by the use of pre and postoperative cathomycin Mange bothered a large number of the animals and contributed to the mortality rate Two hips dislocated one of which went on to apparent union while the other developed aseptic necrosis

SUMMARY AND CONCLUSIONS

When the circulation of the neck of the femur is extensively damaged accurate reposition and fixation will not prevent non union and aseptic necrosis Omental grafts though they bring in circulation carry a high infection potential Vastus lateralis gluteus and other muscle grafts placed in the distal fragment near the fracture site are not as successful as sub capital grafts The most gratifying results have come from the rectus abdominis grafts placed in the head fragment It is the latter variety that we intend to use in our next experimental series

Circulation can be introduced into the heads of fractured femurs aseptic

THE DEVELOPMENT OF CIRCULATION IN THE FEMURS OF DOGS AFTER FRACTURE OF THE FEMORAL NECKS*

CHARLES J. FRANKEL, DAVID V. STRIDER AND W. CLARK POHL

The unsolved fracture so aptly described by Kellogg Speld in 1941 still remains the nemesis of surgeons everywhere. Non union and aseptic necrosis have been attributed by Tovee and Gendron¹ to inaccurate reduction, poor nailing or too early weight bearing. Stuck and Hinchey² concluded that vascular damage was primarily responsible for aseptic necrosis; they devised muscle transplanting procedures which were successful in a few animals but proved to be inadequate for humans.

If Tovee's conclusions and inferences are correct, further research into the problems of introducing circulation into the heads and necks of fractured femurs would be unnecessary. The anatomy of the femoral head and neck of the dog is somewhat different from the human. In the latter there is always a well developed neck which has proven to be an obstinate barrier for circulation to cross in a proximal direction. In dogs and in other animals the trochanteric circulation provides a speedy process of creeping substitution since there is little or no neck intervening. The explanation for Tovee's excellent results, which he attributed to firm and accurate fixation, is probably on the basis of unimpaired circulation. It is completely misleading to draw any inference of badly damaged circulation from a procedure in which the capsule is not adequately cut, the head removed and then replaced and fixed securely. It is necessary to strip the capsule extensively in order to simulate some of the Pauwels Type Three displaced fractures in humans.

Our experiments were undertaken because we became convinced after many years of clinical experience that despite perfect fixation, accurate reduction and adequately delayed weight bearing, non union and aseptic necrosis developed in many cases.³ Cleveland Larsen⁴ and many others have reported the same difficulty despite the use of many different types of fixation materials.

METHOD

Fifty newly acquired mongrel dogs of various ages were operated under intravenous veterinary sodium pentothal anesthesia. Strict aseptic precautions were observed. The right hips were approached through lateral incisions; the hip abductors were sectioned and the capsules were opened by T shaped incisions. The ligamentum teres was sectioned in all but 2 cases. The femoral necks were osteotomized utilizing the Gigli saw and the heads were completely removed from the wounds. Extensive capsular stripping was carried out proximally. The heads were then replaced and accurately fixed by stainless steel screws under direct observation. The capsules and the soft tissues were closed without undue tension and the animals were returned to individual cages for postoperative care.

Group One—Control Group Five Animals. The procedures described above were carried out. All the animals developed aseptic necrosis and non

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METHOD

The data were obtained by review of the case records of all patients with a diagnosis of hip fracture seen at the University Hospitals during the past 15 years. Blood type information and diagnosis were the 2 main factors in the selection of cases. Patients over 60 yr of age with intra and extra capsular fractures resulting from minimal trauma were accepted. Younger patients with similar fractures or patients with fractures resulting from severe trauma were not. The significance of differences in the blood type frequencies of the patient and control groups was tested by the Chi square and difference in percentages methods. Blood donors, all friends or relatives of the patients from the same geographical area with similar genetic anthropologic and ethnic backgrounds were used as controls.

The blood type frequencies of the patient group were also compared with those of a second group of patients of all ages who had incurred various kinds of fractures incident to severe trauma. To control the age factor the blood type frequencies of patients over 60 yr of age receiving consecutive blood transfusions were recorded and compared with those of patients with hip fractures.

In examining the significance of the observed differences 11 different combinations of the 4 blood types are possible. Only the results of the O A and O A B AB comparisons are reported. The other comparisons were made but added nothing of importance to the analysis as has been true in general in these researches. A detailed description of the methods employed in collecting and analyzing the data appears elsewhere.^{2,3}

RESULTS

In Table 1 are recorded the numbers and percentages of the controls and hip fracture patients with each blood type. By examining the differences in the percentage of the controls and patients for each blood type it will be noted that the most striking finding is the increase in blood type A and decrease in O in the patients. Lesser differences in percentage are noted for types AB and B. When the Chi square analysis is made for the O A differences $0.2 < P < 0.5$ indicating significance whereas when the O A B AB differences are examined $10 < P$ indicating no significance. The lack of significance for the second analysis is due to the minor differences in the B and AB frequencies.

Inspection of the data in Table 1 suggests that the evidence for the blood group association is stronger in men than in women. However no evidence of statistical significance $10 < P$ was found for the differences occurring between men and women. Likewise none was found when the blood type frequencies of the male and female patients were separately compared with the controls.

In Table 2 are recorded the data obtained from patients with other kinds of fractures and data from patients 60 yr of age and older receiving transfusions. Comparing the blood type frequencies of hip fracture and other fracture patients $10 < P$ for the O A and O A B AB comparisons. This was also true when the data obtained from men and women were separately examined. For the differences observed between the hip fracture and transfused patients $0.2 < P < 0.5$ for the O A comparison and $10 < P$ for the

necrosis can be prevented and creeping substitution so necessary for the healing of fractures can be speeded up

Further work in which radioactive tracers are used to measure circulation accurately and provide us with an index with which we can prognosticate the future of the damaged bone will be carried out. The inferences that may be drawn from our pilot study are interesting though speculative. We believe that transplants of the rectus muscle in humans will be feasible. We hope we are one step nearer to the solution for the unsolved fracture.

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ON THE ETIOLOGICAL ROLE OF HEREDITY IN FRACTURES*

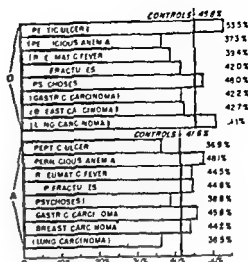
J A BUCKWALTER, J H TURNER, L RATERMAN AND R T TIDRICK

During the past 3 years increasing numbers of investigations have been reported concerning the association of the ABO blood groups and disease. Interest in this field was reawakened by the report of Aird, Fraser, Roberts and Bentall¹ in 1953 indicating a highly significant association between the ABO blood groups and gastric carcinoma in the population of Great Britain.

The results of studies at the University of Iowa Hospitals have in general confirmed those of the British authors and suggested associations in additional diseases. In evaluating these data the blood type frequencies observed in the patient groups have been compared with those noted in a disease free sample of the population referred to as controls. Differences between the blood type frequencies of the patients and controls have been examined for statistical significance. For the purpose of obtaining additional control data patients with hip fractures were studied. The blood type frequencies differed significantly from those of the controls which was not expected. This is a preliminary report of the investigations arising from this chance observation.

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Fig 1 Blood type O and A percentages of controls (blood donors) and patients



the differences between the patients and controls when the O \ B \ AB comparison was made.

The data obtained from patients with other fractures were of interest because of the close agreement of the blood type frequencies with those of the controls thus differing from the hip fracture patients. However because of the small number of patients in the former group no significance was found for these differences. Likewise no significance was found for the differences in the blood type frequencies of the male and female hip fracture patients although inspection of the data suggests the possibility of a difference. Sample size may or may not be responsible for the absence of significance.

Information available with regard to blood type frequencies in persons 60 yr and older is sparse and does not permit a categorical statement to be made that they are the same as those noted in younger persons of the same population. Since blood donors are all younger persons they do not provide an answer to this question. Patients receiving blood transfusions provide a control for the age factor which is admittedly a poor one since their disorders also may be associated with alteration of blood type frequency. Perhaps the best control is provided by comparing the frequencies noted in the hip fracture patients with other patient groups. When this is done highly significant differences between patient groups are noted. In Figure 1 note the differences in blood type O and A frequencies for the various patient groups thus far studied comparing the blood type frequencies of hip fracture and peptic ulcer patients. $P < .001$.

Causality has as yet not been proved for any of the demonstrated ABO blood group disease associations. However, with the accumulation of similar data from various areas of the world for different population groups indicating associations for increasing numbers of diseases an as yet not understood causal relationship related to heredity becomes more an anthropological or specious one less likely. Senile osteoporosis played a causative role of varying importance in the patients with fracture of the hip. The possible relationships of the blood group substances to protein metabolism, endocrine function, and other physiologic processes giving rise to defective bone matrix are at this time matters of sheer speculation.

As suggested earlier life expectancy has not been correlated with the

Table 1 Number and percentage of hip fracture patients and controls (blood donors) with each blood type, and difference in the blood type percentages of the patient and control (blood donor) groups

BLOOD TYPE	PATIENTS									CONTROLS	
	MEN			WOMEN			TOTAL			NO	%
	NO	%	DIFF IN % FROM CONTROLS	NO	%	DIFF IN % FROM CONTROLS	NO	%	DIFF IN % FROM CONTROLS		
O	130	39.6	-6.2	280	42.9	-2.9	410	41.8	-4.0	2892	41.8
A	149	45.4	+3.8	292	44.7	+3.1	441	45.0	+3.4	2621	41.6
B	35	10.7	+1.7	60	9.2	+0.2	95	9.7	+0.7	570	9.0
AB	14	4.3	+0.7	21	3.2	-0.4	35	3.5	-0.1	226	3.6
Totals	328	100.0	0.0	653	100.0	0.0	981	100.0	0.0	6313	100.0

Table 2 Blood types and blood type percentages of patients with other fractures and of transfused patients 60 yr of age and older differences in the blood type percentages from those of hip fracture patients and the controls (blood donors)

BLOOD TYPE	OTHER FRACTURE PATIENTS				TRANSFUSED PATIENTS			
	NO		DIFFERENCE IN % FROM HIP FRACTURE PATIENTS		NO		DIFFERENCE IN % FROM HIP FRACTURE PATIENTS	
O	112	44.4	+2.6	-1.4	810	45.4	+3.6	-0.4
A	103	40.9	-4.1	-0.7	730	41.0	-4.0	-0.6
B	26	10.3	+0.6	+1.3	177	10.0	+0.3	+1.0
AB	11	4.4	+0.9	+0.8	64	3.6	+0.1	0.0
Totals	252	100.0	0.0	0.0	1781	100.0	0.0	0.0

O A B AB No significance was found on examining the data by sex. No significance was found for the differences in the blood type frequencies of the patients with other fractures, transfused patients, and the controls.

The Rh blood type was recorded in 804 of the patients with hip fractures. The frequencies noted in the total patient group, in the patients with type O, A, B, and AB blood, and in male and female patients did not differ significantly from the Rh frequencies observed in the controls.

DISCUSSION

The evidence presented suggests an association between the ABO blood groups and hip fractures. This is characterized by a significantly higher frequency of blood type A and lower frequency of blood type O in patients with these fractures, or there was a significantly higher incidence of this lesion in persons with blood type A and lower in those with blood type O. The incidence in persons with blood types B and AB did not differ from the expected as indicated by the failure to find statistical significance for

Plastic Surgery

MODIFICATION OF HOST HOMOGRAFT TISSUE INTERACTIONS IN SEPARATED PARABIONTS*

RICHARD H. ANDRESEN CLARENCE W. MONROE DOROTHY A. MADDEN
AND CLORF M. HAY

Previous studies of rabbits united in parabiosis by the rural method showed normal sequences of healing along the line of surgical union. Three to 4 days following anastomosis, a cross circulation was established between parabionts. Epithelial continuity was attained and a mutual deposition of collagen and reticulum occurred in a manner so as to tightly adhere the homologous surfaces together. In 7 to 10 days after anastomosis a characteristic inflammatory reaction developed which appeared to interfere with the dermal continuity and the cross circulation. In spite of the intense inflammatory reaction and the ischemia along the line of anastomosis the aural junctions remained well apposed for as long as 5 mo. in some experiments. Apparently the mutually deposited collagenous matrix was resistant to the autolytic mechanisms operating in most inflammatory processes.¹

Additional studies showed that if parabionts once separated from one another were rejoined cross circulation was rarely established and the aural junctions usually separated spontaneously by the 5th or 6th day. This observation raised a question as to the nature of difference between parabionts following the initial period of parabiosis.

In an attempt to further characterize the effect of parabiosis the following experiments were designed. It seemed reasonable to assume that such differences might become apparent through a critical analysis of the host graft tissue interactions under aseptic circumstances such as is the case with musculofascial grafts.

METHOD

Thirty pairs of New Zealand rabbits were surgically united (rural method) for 13 to 15 days and then separated by amputation of the joined ears under 1 per cent procaine solution. Ten to 21 days after separation 15 pairs were reunited in a similar manner and again separated. The 15 remaining pairs were united once and separated after 10 to 21 days.

At intervals of 6 to 90 days following separation grafts of erector spinae muscle measuring $2.0 \times 1.5 \times 0.5$ cm. with attached fascia were resected under sterile precautions and cross transplanted between respective parabionts. Control autografts were also made. Two weeks following transplantation, the animals were sacrificed via cisterna puncture with 1 per cent procaine solution. The skin was removed from the backs and the animals

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ABO blood groups It is conceivable that those persons with group A have shorter life expectancy than group O and both shorter than group B. These matters will have to be settled by extensive observations and careful statistical evaluation.

Confirmation or rejection of the suggested association and the answers to the questions concerning sex and age should be provided by the collection of additional data. However, the answer to the question concerning causality will be more difficult to obtain. In addition to similar data collected from different racial and population groups, appropriate basic investigations designed by geneticists, biochemists, endocrinologists, enzymologists and clinicians will be required.

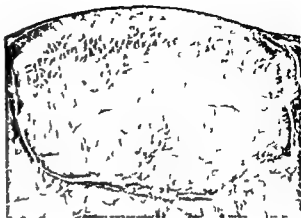
SUMMARY

The ABO blood type frequencies of patients with hip fractures have been compared with those of blood donors with patients with miscellaneous fractures and with transfused patients 60 yr of age and older. Evidence of statistical significance was found suggesting an association between the ABO blood groups and hip fractures characterized by an increased incidence of this injury in persons with blood type A. These findings suggest a possible role for heredity in the etiology of hip fractures.

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Fig 2 This is a low power photo micrograph (Magnification $\times 10$) of a postparabiotic musculofascial cross homograft 2 wks of age. This graft is similar to that shown in Fig 1 with the exception that the fascia and area beneath contain a peculiar angiomatous pattern of patent vascular channels. In spite of this rich vascularization collagen deposition and fibroblastic proliferation are curtailed. Inflammatory cell infiltrate and angitis are not observed. This represents the second form of postparabiotic host homograft tissue interaction and is found in one half of the group of separated rabbit parabionts.



the graft remained delayed and there was no tendency to elicit the characteristic inflammation usually seen in homografts (Fig 2)

DISCUSSION

The present studies have shown that the host homograft tissue interaction in the postparabiotic period is highly specific and readily reproducible between parabiotic partners for as long as 90 days. This type of host graft tissue interaction is different from those previously described.² It appears to resemble more closely the host graft tissue interactions obtained when homologous musculofascial grafts are treated at low temperatures or with irradiation *in vitro* prior to transplantation.

In order to determine whether this modification was applicable to other types of homografts a series of skin homografts was made between separated parabionts. These experiments have disclosed that the skin homografts made between separated parabionts in the postparabiotic period are remarkably well tolerated. The grafts do not slough away as is customary for skin homografts in a period of about 2 wks. On the contrary, the grafts persist though they slowly shrink in dimensions, finally disappearing over a period of many weeks. The appearance of these grafts at the end of 9 weeks is shown in Figure 3.



Fig 3 This is an illustration (Natural Size) showing 2 skin cross homografts in a separated rabbit parabiont 9 wks after transplantation. Note that the grafts after this period of time remain sharply demarcated and are well healed in place. The grafts appear to be well tolerated by the host.

placed in 10 per cent formalin solution. Following fixation the grafts were sectioned in a plane perpendicular to the spinal column. The tissues were embedded in paraffin cut and stained with hematoxylin and eosin.

RESULTS

Cross examination of the musculofascial grafts at 2 wks of age showed that they were well healed in place. A bursal space had formed over both types of grafts. The autologous musculofascial grafts showed a red heavy pannus of vascularized granulation tissue which had grown over the fascia of the grafts. On the other hand, the parabiotic homografts showed gradations from a pearly white appearance to one showing pink streaking indicative of the varying degrees of vascularization of the pannus by the host.

Microscopic examination of the autologous musculofascial grafts in separated parabionts at 2 wks of age revealed the customary pattern of tissue reaction which consisted of orderly sequences of degeneration and organization in the absence of inflammation.

Microscopic examination of the parabiotic homografts cross transplanted between respective parabionts showed 2 types of host-graft tissue interactions which were about equally distributed between the 2 groups of separated parabionts. The first type of reaction was characterized by an avascular collagenous encapsulation of the graft. There was delayed absorption of the graft though it was well healed in place. The fascia of the graft remained normally cellular and was covered by an avascular pannus of mesenchyme derived from the host. The muscle bundles remained in close approximation to the fascia so that absorption was taking place principally from the lateral and inferior aspects of the graft. Inflammation was not observed although the muscular tissues of the host adjacent to the graft often contained foci of plasma cells (Fig. 1).

The second type of tissue reaction in separated parabionts was similar to the first except that vascular penetration did occur. This consisted of the development of a peculiar angiomatous pattern of patent blood vessels beneath the fascia of the graft. In spite of the intense vascularization collagen deposition and fibroblastic activity did not appear. Also absorption of

Fig. 1 This is a low power photomicrograph (Magnification $\times 40$) of a postparabiotic musculofascial cross homograft 2 wks of age. The empty space above the tissue represents the bursal space which usually forms above all types of musculofascial grafts in rabbits. The floor of the bursal space is formed by a delicate pannus of avascular mesenchymal tissue which has originated from the host. Beneath the pannus is the fascia of the graft which is somewhat thickened due to retraction. The muscle bundles of the graft remain well approximated to the overlying fascia. The architecture of the graft is unchanged. This host-homograft tissue interaction represents one form encountered in parabiotic pairs during the postparabiotic period.



cific antibodies. Since the properdin system is a new and unexplored phase of the immune response, it appeared to offer a feasible field for the study of transplantation of tissues. This was of particular interest since the properdin system can be altered by the administration of certain agents (Zymosan, dextrans, levamis and other carbohydrate complexes) thus allowing controlled host properdin responses to the transplantation of tissues.

To date the best evidence for failure of renal homotransplants appears to be a function of the host's immune response although a specific blood born anti-tissue antibody has not yet been detected by standard serological techniques.⁴ It has been postulated that suppression of this immune response would lead to successful homografts and many efforts have been directed towards this goal in the past.^{5, 6, 7}

Therefore renal homotransplantation was undertaken to see what effect the depression of the properdin system would have on survival of homotransplants.

METHOD

The experimental laboratory animals utilized in this study were healthy mongrel dogs. The serum from 10 cc of clotted blood was assayed for initial properdin levels in each of 19 animals. The properdin titers were determined by the standard technique described by Pillmer¹ and performed in his laboratory. Animals with titers above 2 units/ml were suitable for Zymosan administration (Zymosan is a suspension of insoluble cell wall residue from yeast). The LD₅₀ was approximated in a preliminary group of 6 dogs by the intravenous administration of Zymosan suspension.

The animals were anesthetized with 1% sodium pentobarbital 30 mg/kg and then Zymosan was injected intravenously in dosages varying from 5 to 20 mg/kg body weight. In 2 animals renal transplantation from donor dogs was done immediately. In 6 animals the Zymosan was administered 24 hr prior to transplantation (Fig 1).

After suitable exposure of the carotid and jugular vessels the carotid artery of the host was sutured to the renal artery of the donor and the jugular vein to the renal vein with 5-0 silk. The ureter was then brought out through a stab wound in the neck. A suitable pocket was formed in the neck for the donor kidney which was fixed *in situ* by sutures to prevent

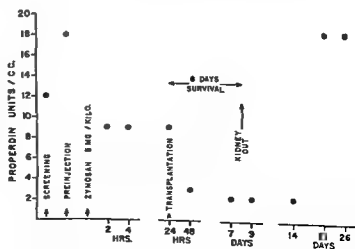


FIG 1 (U 7) Showing usual time relationship of properdin levels Zymosan administration and renal homotransplantation. Transplantation done 24 hr after Zymosan. Properdin level low for about 14 days. Graft survived 11 days during period of low properdin levels.

SUMMARY

Rabbits were united in parabiosis by the rural method for varying periods of time and then separated. From 6 to 90 days following separation musculofascial grafts were cross transplanted between specific parabiotic partners. Two weeks after transplantation the grafts were prepared for microscopic study. This revealed 2 types of host graft tissue interactions. The first type consisted of an avascular collagenous encapsulation of the graft. The architecture of the graft remained unaltered. Inflammation did not occur. The second type of host homograft interaction in separated parabionts was similar to the first except that vascular penetration was conspicuous. A curious angiomatous pattern of vascular channels developed beneath the fascia of the graft. In spite of the intense vascularization collagen deposition and fibroblastic proliferation did not take place.

Other tissues such as skin cross homografts were studied in the post parabiotic period. Skin homografts under these conditions were well tolerated by the hosts for extended periods of time. A method has been described by which the inflammatory aspects of the host homograft tissue interaction between 2 animals of the same species has been entirely eliminated.

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PRELIMINARY EXPERIENCE WITH PROPERDIN AND RENAL HOMOGRAFTS*

CHARLES A. HUBAY, LESTER PERSKY AND WILLIAM D. HOLDEN

The properdin system described by Pillemer^{1, 2, 3} and associates has been shown to be an integral part of the natural defense mechanism of blood. Properdin has been felt to be separate and distinct from immune mechanisms involving antibody antigen reactions. This serum protein is a euglobulin with a molecular weight 8 times that of gamma globulin and represents not more than 0.03 per cent of the total proteins. It is dependent for its action upon complement and Mg^{++} and is active against certain bacteria and viruses and lyses certain abnormal erythrocytes in the absence of specific

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Table 1 Summary of results of renal homotransplantation in 8 experimental animals

ANIMAL	U6	U7	U3,	U11	U18	U19	U9	U61
ZYMOSAN MG/KG	5	5	25	5	10	10	5	5
TIME OF TRANSPLANT	21 hrs	21 hrs	imme- diate	21 hrs	21 hrs	imme- diate	21 hrs	21 hrs
GRAFT SURVIVAL	7 days	8 days	1 day	8 days	1 day	2 days	8 days	8 days
INFECTION	+	+	++++	+++	++++	+++	+++	+++

Table 2 Properdin levels in experimental animals before and after Zymosan administration. Note return to normal levels in animals surviving 2 or more wks

ANIMAL PROPERDIN TITERS	U6	U7	U3,	U11	U18	U19	U9	U61
Preliminary	12	12	16	9	18	21	12	12
Reinjection	18	18	12	5	10	18	2	2
1 hr	18		2	1	10	12	3	2
2 hr	18	9	2	5	2	6	1	2
4 hr	18	9	2	5	2		4	2
21 hr	18	9	2	■	2	1	2	2
18 hr	6	3						
72 hr	3		2		■	1	2	2
1 wk	3	3	2			18	2	2
9 days	3	3						
10 days	3			2			2	■
2 wk	21	3	2			20		1
3 wk	21	18						
96 days	18	18						
1 wk	18							
Fate	Sic	Sic	Died	Sic	Died	Sic	Sic	Sic

It should be pointed out that control studies of the effect of Zymosan on the properdin system in dogs would have been desirable. This was not feasible however owing to the small number of dogs suitable for Zymosan administration (22.5 per cent of dogs screened).

Although these preliminary experiences with renal homografts and the properdin system were not successful we feel that this type of study should be continued with more suitable dogs maintained under optimum conditions or alternatively with another species of experimental animal whose properdin level is less labile and who is innately more resistant.

SUMMARY

The properdin system was explored in dogs to determine its role in the response of recipient animals to renal homotransplants. When the properdin system was artificially depressed homotransplantation was not favorably

The authors wish to express their appreciation to Dr Louis Lillemer whose counsel and laboratory aid made this study possible and to Dr Simon Kolketsky for microscopic examination of the transplanted kidneys.

rotation and linking of vessels. The subcutaneous tissues and skin were then closed with 4-0 silk. A small polyethylene catheter was then inserted into the ureter to the renal pelvis and sutured to the skin. This allowed daily saline irrigations to remove clots and insure against linking of the ureter.

Additional properdin titers were drawn at periodic intervals after the administration of Zymosan.

RESULTS AND DISCUSSION

Forty-nine animals were screened and initial properdin titers obtained. Of these animals only 11 had titers which were deemed adequate for Zymosan administration. For technical reasons 3 of these animals could not be used. This over all percentage of 22.5 suitable levels reflects the general undesirability of using mongrel dogs in experiments involving the properdin system. The titers may be low owing to chronic infestations and infections which are so often found in pound obtained dogs. This observation has been substantiated by the experience of Fine⁹ and Michelson (personal communication to Pillemier) who found that the proper care of inbred beagle dogs result in consistently normal properdin titers. An alternative would be the employment of a different experimental animal. In a study such as renal homotransplantation however the size of the renal vessels in smaller animals precludes their use routinely.

In the preliminary determinations of the approximate LD₅₀ it was found that dosages over 25 mg/kg of Zymosan saline suspension intravenously resulted in the death of the animal within 24 hr. The administration of Zymosan in these doses was accompanied by vomiting, severe tenesmus, bloody diarrhea, wheezing respirations and rapid death. Therefore Zymosan dosages of 5 to 10 mg/kg were used giving satisfactory depression of properdin levels.

The length of survival of the homografts is illustrated in Table 1. Survival varied from 24 hr to 8 days. The death of the kidney was attended by diminution of urine output and hematuria. Microscopic examination of the kidneys showed typically severe pyelonephritis.

Properdin titers were depressed by administration of Zymosan and these are shown in Table 2. One of the difficulties encountered in this study was the spontaneous fall of the properdin level between the screening and the subsequent Zymosan injection and operative procedure. This period was approximately 2 wks. during which time the animal was maintained under routine kennel care.

Death in 2 animals (U35-48) appeared to be from overwhelming septicemia despite postoperative penicillin and streptomycin therapy. The remaining animals were given oral neomycin 1/0 days for 2 days prior to transplantation and received postoperative penicillin streptomycin therapy. Nevertheless these animals also developed non fatal infections at the transplantation site.

The relationship between the hypoproperdinemia and the bacterial infections noted in these animals has been substantiated by the work of Wardlaw and Pillemier⁸ who demonstrated that the removal of properdin from serum also removes bactericidal activity. The addition of properdin to properdin-deficient serum restored bactericidal activity.

METHOD

Five pairs of mongrel dogs were used maintaining the same donor recipient relationship throughout. Under nembutal anesthesia a 10×5 cm recipient area on a shaved portion of the chest wall was outlined with a scalpel. The skin was removed down to the level of the panniculus carnosus by blunt dissection requiring fairly strong traction. In this manner the plane of cleavage was easily found and bleeding minimized. Different recipient areas were used for each operation. Split thickness grafts of donor skin were cut with a thin bladed knife, several pieces being used to cover the area. Control autografts were applied in a number of instances. The grafts were covered by fine mesh gauze and fixed in position by cotton waste and adhesive tape, the whole area being protected by a calico jacket.

The grafts were inspected daily from the fourth day, the survival time being judged by gross appearance. Serial color photographs were invaluable in retrospective assessment of rejection. Serial biopsies were taken where practicable. No dressing was required after the seventh day. The time interval between grafts varied from 7 to 55 days and the total number of operations for each pair of animals ranged between 3 and 10.

RESULTS

At the first dressing after the first operation the graft was well vascularized in each instance. There was slight epithelial outgrowth from the edges of the graft. Subsequently the whole area became completely epithelialized, the graft becoming supple (Fig 1a and b). The first sign of rejection was a color change of the graft from normal to dark red and finally to black. These changes occurred over a period of 21 to 48 hr, followed rapidly by complete sloughing of the graft. Reepithelialization occurred from surrounding host epithelium or from the control autograft, leaving this recipient bed contracted.

After the later serial operations the appearance of the graft on first inspection was very different. In some instances the soft swollen and moist graft demonstrated no evidence of epithelial outgrowth at its margins and could be detached easily from its bed (Fig 2a and b); in others only islands of the transplanted skin were present and the remaining area consisted of friable granulations which bled readily, evidence of a marked homograft

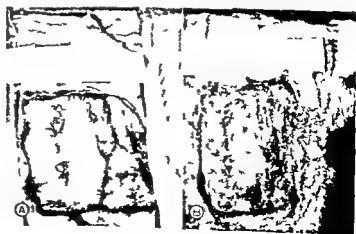


Fig. 1. Appearance of first set split thickness skin homograft (a) at operation and (b) on the 5th day showing survival and epithelial proliferation (Dog #3).

influenced. It is pointed out that mongrel dogs under average kennel conditions are unsuitable for this type of experiment owing to the high percentage with initially low properdin titers.

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A STUDY OF CONSECUTIVE SKIN HOMOGRAFTS IN THE DOG*

LEWIS THOMAS AND JOSEPH E. MURRAY

An attempt has been made to modify the homograft reaction in the dog by the application of consecutive skin grafts from the same donor to the same recipient. Medawar¹ using rabbits demonstrated that the second homograft from the same donor is rejected in a shorter time than the first and postulated an antigen-antibody reaction as the basis of the rejection. This has been the current working hypothesis for most studies of homotransplantation. An increased percentage of permanent survival of homografts after a series of skin grafts on heterozygous CFW mice has been described by Werder and Hardin, presumably by overwhelming the immune reaction by the repetitive doses of antigen.² The present study is an attempt to apply this principle of immunoparalysis to an animal with genetic and immunologic characteristics more nearly resembling man.

As the homograft problem in man is being more fully investigated and understood it appears that patients with chronic uremia can tolerate homografts of skin and kidney longer than normal recipients. If a further lowering of the immune response can be obtained in such recipients by serial applications of antigen the clinical usefulness of renal homotransplants might be expanded.

From the Surgical Service, Peter Bent Brigham Hospital and the Laboratory for Surgical Research, Harvard Medical School, Boston, Mass. Supported by a grant from the United States Public Health Service. The assistance of the Morgan Williams Request Fund, University of Wales is also gratefully acknowledged.

METHOD

Five pairs of mongrel dogs were used maintaining the same donor recipient relationship throughout. Under nembutal anesthesia a 10×5 cm recipient area on a shaved portion of the chest wall was outlined with a scalpel. The skin was removed down to the layer of the panniculus carnosus by blunt dissection requiring fairly strong traction. In this manner the plane of cleavage was easily found and bleeding minimized. Different recipient areas were used for each operation. Split thickness grafts of donor skin were cut with a thin bladed knife, several pieces being used to cover the area. Control autografts were applied in a number of instances. The grafts were covered by fine mesh gauze and fixed in position by cotton waste and adhesive tape, the whole area being protected by a calico jacket.

The grafts were inspected daily from the fourth day, the survival time being judged by gross appearance. Serial color photographs were invaluable in retrospective assessment of rejection. Serial biopsies were taken where practicable. No dressing was required after the seventh day. The time interval between grafts varied from 7 to 50 days and the total number of operations for each pair of animals ranged between 3 and 10.

RESULTS

At the first dressing after the first operation the graft was well vascularized in each instance. There was slight epithelial outgrowth from the edges of the graft. Subsequently the whole area became completely epithelialized, the graft becoming supple (Fig. 1a and b). The first sign of rejection was a color change of the graft from normal to dark red and finally to black. These changes occurred over a period of 24 to 48 hr, followed rapidly by complete sloughing of the graft. Reepithelialization occurred from surrounding host epithelium or from the control autograft leaving this recipient bed contracted.

After the later serial operations the appearance of the graft on first inspection was very different. In some instances the soft swollen and moist graft demonstrated no evidence of epithelial outgrowth at its margins and could be detached easily from its bed (Fig. 2a and b); in others only islands of the transplanted skin were present and the remaining area consisted of friable granulations which bled readily, evidence of a marked homograft



Fig. 1 Appearance of 1st set split thickness skin homograft (a) at operation and (b) on the 5th day showing survival and epithelial proliferation (Dog #3)

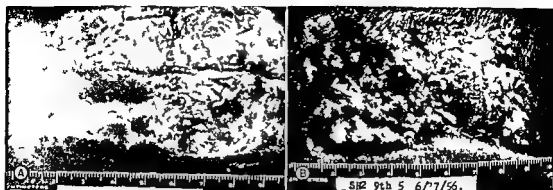


Fig 2 (a) 8th set homograft on the 6th post operative day (Dog #2) (b) 9th set homograft on the 5th post operative day (Dog #2) Note edematous indolent appearance of grafts (Contrast to Figure 1b)

reaction (Fig 3) Occasionally infection secondary to rejection made assessment of survival difficult. Most autografts survived and finally increased in size to cover the defect left by the sloughing homograft. In 2 instances autograft failure was due to infection.

The survival time of the first set graft ranged from 6 to 15 days with each consecutive set the time became progressively shorter to about 4 days on the third set. With these later grafts there was never any epithelial proliferation edema was prominent and the area was hyperemic further evidence of the homograft reaction. At no time even after 10 consecutive grafts was there any evidence of prolonged survival of the skin transplanted. Instead each graft was rejected in a shorter time than the initial graft of the series (Table 1). Prolongation of the interval between grafts increased the period of survival to a slight degree. In Dog #3 after a 45 day interval graft survival time increased from 4 days to 6 but still did not approach the 9 day survival period of the first set graft (Fig 4). This slight increase in survival time after the 45 day interval may not appear significant in time but the gross appearance of the graft indicated a less severe homograft reaction. The outline and substance of the graft persisted whereas the earlier graft was completely destroyed at the fourth day. All data are tabulated in Table 1. In Figure 4 the data for dogs #2, 33 and 38 are charted in order to indicate more graphically the variations in survival time related to the intervals between grafting.

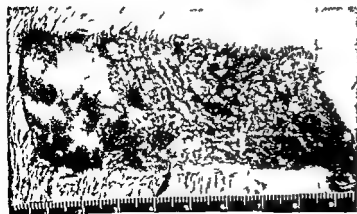


Fig 3 2nd set homograft at 7th post operative day. Small areas of homograft remain but are black and will slough in 24 to 48 hours. An autograft is surviving at the right margin (Dog #9)

Table 1 Survival times of serial skin homografts in dogs

OPERATION	DOG 1		DOG 2		DOG 3		DOG 8		DOG 9	
	INTER	DAYS	INTER	DAYS	INTER	DAYS	INTER	DAYS	INTER	DAYS
	VAL	OF	VAL	OF	VAL	OF	VAL	OF	VAL	OF
	BETWEEN	GRAFT	BETWEEN	GRAFT	BETWEEN	GRAFT	BETWEEN	GRAFT	BETWEEN	GRAFT
OPERATIONS	OPERATIONS	SURVIVAL	OPERATIONS	SURVIVAL	OPERATIONS	SURVIVAL	OPERATIONS	SURVIVAL	OPERATIONS	SURVIVAL
1st	—	8	—	10	—	9	—	6	—	7
2nd	17	6	25	7	11	1	20	1	30	7
3rd	11	1	27	7	7	1	16	1	50	1
4th	15	10110	11	1	17	1	10	11		
5th			7	1	7	1	30	1		
6th			1	5	11	1	10	1		
7th			17	6	15	1				
8th			10	7	10	6				
9th			36	5	11	1				
10th					37	1				

DISCUSSION

This investigation has been one phase of a clinical and experimental study of the homotransplantation of kidney and skin. The dog rather than inbred strains of mice and rabbits has been selected as the experimental animal because their genetic and immunologic characteristics more nearly resemble man and make results more useful. Most experimental data derived from the lower animal probably should be tested in the dog prior to clinical application.

In order to overwhelm the immune mechanism the maximum antigenic response is desirable. Therefore large areas of skin were grafted because Medawar¹ has demonstrated that smaller homografts possess a lower dose of antigen and therefore survive longer than large ones.

The results seem definite: there is no increase in the survival of skin homografts in the dog following the application of serial homografts. There is no evidence that the immune mechanism is overwhelmed or the antibody response lowered by repetitive doses of antigen.

SERIAL SKIN HOMOGRAFTS

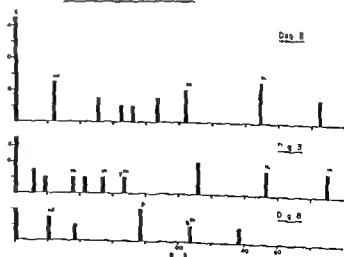


Fig. 4 Average survival times of skin homografts for dogs #2 #3 #8 indicating accelerated rejection after 1st operation.

Note the relative increase of survival if interval between operations is prolonged.

There is evidence that the accelerated rejection of a second set homograft is not permanent. It can be modified by the subsequent plan of experimentation. After a lapse of 15 days the immune reaction diminishes as evidenced by a slight prolongation of survival of the graft. However in no animal did any subsequent graft even after a 55 day interval survive longer than the original graft. This may contrast with the observations in mice of Iehrsfeld, Taylor and Converse that second homografts applied 60 days after the first survived as a primary graft and did not demonstrate the second set phenomenon. Either there is a species difference or a longer interval is required to eradicate the heightened immunity in the dogs. In our present series more grafts will be applied at longer intervals to determine the length of survival of this accelerated reaction.

CONCLUSIONS

The results support the immune mechanism theory of homograft rejection, the state of heightened immunity being shown by the more rapid rejection of each set of consecutive homografts. The homograft survival was not prolonged by serial homografting. This method does not seem suitable for clinical trial.

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THE FATE OF THE SKIN HOMOGRAFT IN THE CHRONICALLY UREMIC PATIENT*

NATHAN P. COUCH, JOSEPH F. MURRAY, GUSTAVE J. DAMMIN AND
IRVING P. THOMAS

Because in immune response underlies rejection of tissue homotransplants in man and the experimental animal, studies of both naturally occurring and artificial means of suppressing immune mechanisms have special meaning for all surgeons concerned with tissue grafting.

*Aided by the United States Public Health Service, John A. Hartford Foundation, and the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

With the assistance of Dr. John Merrill, Dr. Warren Cuild, and the Kidney Laboratory of the Peter Bent Brigham Hospital.

Homovital (as opposed to *homostuctural*) tissue transplantation has proven value in chronic renal failure in kidney transplantation between identical twins where the recipient has chronic uremia "virtual cure can be achieved." Even when the chronically uremic recipient receives a renal homograft from an unrelated donor homotransplant function is sometimes maintained for 5 to 25 wks. In these patients with widely varying types of renal disease chronic uremia is the outstanding common denominator. In the normal dog renal homograft function usually fails before 1 week⁷ and in man where renal homografts have been performed for acute rather than chronic renal failure function ends by 3 weeks.¹⁰ In some chronically uremic patients then renal homograft survival appears to be prolonged. Since antigenic kinship between skin and kidney has been demonstrated in the dog it seemed plausible and worthy of experimental trial that homografts of skin might also enjoy extended survival in the presence of chronic uremia.

The course of vascularization and rejection of the human skin homograft in the normal recipient is constant with eschar formation by day 15 and sloughing by day 20 leaving a dermal pad in the host bed.⁸ Gibson and Medawar⁹ observed that epidermal degeneration in skin homografts was grossly complete by 23 days while in contrast the dermal collagen persisted for at least 36 days and underwent destruction before 60 days.

Our objective in the present study was to determine whether there is in fact prolonged skin homograft survival in patients with chronic renal failure.

METHOD

Ten patients were used. These included 6 patients (Group A) with chronic uremia due to differing forms of renal disease: chronic pyelonephritis, chronic glomerulonephritis, and polycystic kidneys. The durations of uremia varied from 1 mo. to 6 yr. while ages ranged from 20 to 62 yr. Skin donors were obtained without attempt to secure blood group compatibility between donor and recipient. In 2 cases grafting was between opposite sexes. Three patients received more than 1 homograft. A total of 4 homografts came from donors on long term cortisone treatment, 2 from other chronic uremic patients, and 5 from metabolically normal persons. Three other patients (Group B) also uremic received similar homografts from their healthy identical twins. Finally a control male adult received 2 normal skin homografts.

Homografts and control autografts were placed on the recipients' upper extremities. The homografts were of split thickness; the autografts were of full thickness and the recipient beds were subcutaneous tissues. All grafts were 2 to 3 cm. square and were fitted and sutured to adjacent recipient skin. Gross observations and photographs were made at frequent intervals beginning no later than the seventh day after grafting.

Most grafts were biopsied once only, the biopsy dates ranging from post-grafting day 32 to day 115. Specimens were fixed in 10 per cent formalin, processed through modified Bouin solution and stained with hematoxylin-eosin, periodic acid-Schiff, Verhoeff-van Gieson, reticulum, and Feulgen stains.

Our histologic estimate of *epidermal* survival is still uncertain. It is theoretically feasible to distinguish between donor and recipient epidermis when

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Because an immune response underlies rejection of tissue homotransplants in man and the experimental animal, studies of both naturally occurring and artificial means of suppressing immune mechanisms have special meaning for all surgeons concerned with tissue grafting.

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uremic donors showed no consistent differences from the normal homografts. The homografts between identical twins (Group B) were all graded excellent while those placed on the normal control underwent frank rejection by 13 days and eventual scarring.

The results in the 6 chronic uremic patients and 1 identical twin are outlined in Table 1.

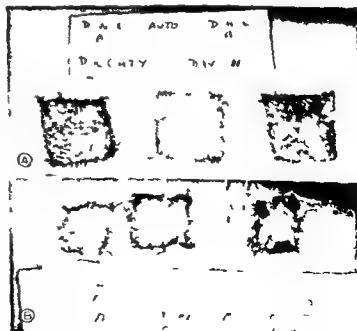
In Figure 1 two homografts undergoing rejection at day 13 in the control patient (Fig 1a) are compared with the day 21 surviving homografts (Fig 1b) in a uremic patient (MS). While the rejected homografts are dark brown coarse thickened dry wrinkled and underlain by yellowish exudate the surviving homografts retain normal pink color normal texture and normal rectangular shape without exudation.

In Figure 2 a perfectly surviving homograft between identical twins is compared histologically with a healed rejection site in a chronic uremic patient (from a later study) at day 12 (rejection completed at day 11) a surviving homograft in a uremic patient (J1) at day 33 and a second surviving homograft in another uremic patient (DB) at day 115. While the rejected graft site showed absence of rete pegs almost complete loss of clusters dense fibroplasia and only scattered dermal appendages the latter two grafts maintained fairly normal cluster pattern intact collagen minimal inflammation and dermal appendages of surprisingly normal appearance.

DISCUSSION

Although these studies are incomplete survival of dermal elements appears to be prolonged in varying degrees in some chronic uremic patients. Our evidence for similar prolongation of epidermal survival is based on gross criteria. If the graft epidermis is slowly and insidiously replaced by ingrowth of host epidermis the absence of the classical gross features of epidermal rejection in homografts of this size is still a notable departure from the normal.

Fig 1 (1a) From left to right homograft autograft and homograft on a normal control at day 11. Darkening thickening and exudation are apparent and indicate early phase of rejection. (1b) From left to right autograft pure homograft raw surface and homograft from a continuous treated patient at day 21. Except for necrosis of control one homograft around sutures both homografts appear similar to autograft.



the 2 are of opposite sex by the chromocentor count method.¹¹ This technique depends on the fact that the female sex chromatin which is larger than the male chromocentor and visible at the nuclear periphery appears in a much higher percentage of epidermal cells in the female than in the male. In 2 such cases, the differential counts were unconvincing. (Methods for similar counts on whole cells garnered from epidermal scrapings and stained by cytologic methods may be more satisfactory.)

Our evaluation of dermal survival depends on persistence of its appendages (hair follicles, sweat glands, smooth muscle, and sebaceous glands), the patterns of the elastic and collagen, the subepidermal (PAS positive) mucopolysaccharide, and the degree and type of inflammatory cell infiltration—all components separated from the recipient tissue by a sheet of dense scar.

RESULTS

Survival ratings were arbitrarily graded: poor, fair, good, and excellent, since the survivals did not follow an all or none principle. Of the 11 homografts in the first 6 uremic patients (Group A) 1 were rated poor, 2 fair, and 5 good. In all 7 grafts rated fair or better at no time was there gross evidence of rejection. The homografts from cortisone treated and

Table 1. Survival of Skin Homografts

RECIPIENT	SURVIVAL OF SKIN HOMOGRAFTS				SURVIVAL
	DONOR	HOMOGRAFT	INTERVAL		
MS 53 F A+	RD	M	Ure	32 d	Good
		A+			
	RJ	M	Cortisone	32 d	Poor
JI 27 M O+	II 57 M		Cortisone	33 d	Good
		A+			
	JN 22 M		Ure	33 d	Poor
		O+			
RH 23 M O+	AK 62 M		Uremic	33 d	Infection
		B+			
	RH 23 M		Ure	37 d	Excellent
		O+			
AK 62 M B+	II 57 M		Cortisone	57 d	Fair
		A+			
	JN 22 M		Ure	57 d	Poor
		O+			
JI 27 M O+			Uremic	57 d	Fair
		O+			
JW 20 M O+	MS 59 F		Ure	61 d	Good
		O+			
RM 26 F O+	MM 19 F		Cortisone	117 d	Good
		O+			
DB 33 M A+	WI 52 M		Ure	115 d	Good
		O+			

All recipients are from Group A (see Methods) except RH who received a homograft from an identical twin and is included for comparison.

terms of tissue homografting as well as our constant need for testing the applicability of animal data to the human this method of altering the immune response is under further investigation in the chronically uremic human

SUMMARY

Prolonged survival in varying degrees of skin homografts was observed in some patients with chronic renal failure. This accords with the occasional finding of prolonged function of renal homotransplants in similar patients. Studies of additional normal control recipients by the same methods are in progress.

We have not found abnormalities in the various aspects of the immune mechanism so far investigated but additional factors including antigen antibody combinations and the properdin magnesium complement system are under scrutiny.

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THE EFFECT OF SKIN CYCLES ON THE SURVIVAL TIME OF SKIN HOMOGRAFTS IN MICE*

IRA M. DUSHOFF AND PETER RANDALL

Considerable experimental work has been done in an effort to extend the survival time of skin homografts. Rodents have been chiefly used and despite use of inbred strains there has been notable variation in the reported experimental results both within single experiments and between different experiments making interpretation of the data quite difficult. In all of this

From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania.

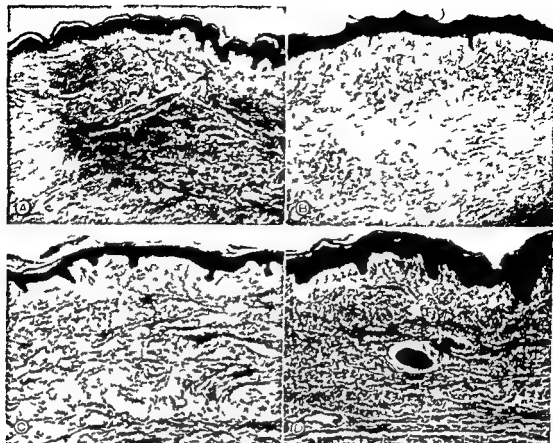


Fig 2 All Verhoeff van Gieson elastic tissue stains 2a—Case R H Homograft from identical twin donor at day 37 near junction of graft and recipient tissue. Note normal rete pegs elastic tissue and collagen patterns and persistence of smooth muscle $\times 128$ 2b—Case R I Site of rejected homograft in chronic uremia at 12 days. Note absence of rete pegs monotonous collagen pattern and numerous capillaries. Only a few strands of elastica remain in upper dermis $\times 128$ 2c—Case J F Skin homograft in a chronically uremic patient at day 33. Note scattered dermal inflammatory cells, good rete pegs and normal elastic tissue and collagen patterns $\times 175$ 2d—Case D H Skin homograft in a chronically uremic patient at day 115. The rete pegs elastica and collagen patterns are preserved. Graft recipient junction is at lower border $\times 175$

It must be stressed that completion of this study awaits evaluation of similar homografts in several additional normal control human recipients.

The reason for this protracted survival are obscure. Preliminary studies in several chronically uremic patients of serum gamma globulin, serum paper electrophoretic patterns, skin tuberculin and Schick tests, isoagglutinin titers and response to blood group specific substances have revealed no abnormalities. Furthermore, others have found no significant changes in complement levels attributable to acute uremia¹ or chronic uremia.⁷ Elevations of the alpha 2 globulin have been recently described in uremia.⁸

Pillemer and Hinz¹⁰ have demonstrated depressions of properdin levels in a small number of uremic patients. One of these—a patient with glomerulonephritis—showed a rise in properdin as his uremia subsided.

Considerable data from animal experiments indicate that immune responses may be altered by administration of various tissues prior to transplantation of the same or related tissues. Blood elements have been extensively studied in this regard.¹¹⁻¹³ Because of therapeutic implications in

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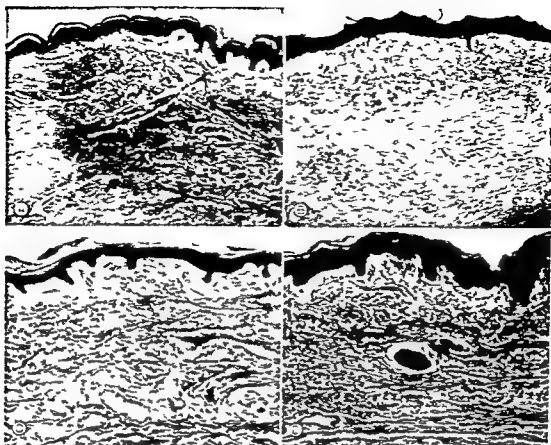


Fig 2. All Verhoeff van Gieson elastic tissue stain. 2a—Case R.H. Homograft from identical twin donor at day 3¹ near junction of graft and recipient tissue. Note normal rete pegs, elastic tissue and collagen patterns and persistence of smooth muscle. $\times 128$. 2b—Case R.L. Site of rejected homograft in chronic uremia at 42 days. Note absence of rete pegs, monotonous collagen pattern and numerous capillaries. Only a few strands of elastica remain in upper dermis. $\times 128$. 2c—Case J.F. Skin homograft in a chronically uremic patient at day 3¹. Note scattered dermal inflammatory cells, good rete pegs, and normal elastic tissue and collagen patterns. $\times 175$. 2d—Case D.H. Skin homograft in a chronically uremic patient at day 11¹. The rete pegs, elastica and collagen patterns are preserved. Graft recipient junction is at lower border. $\times 175$.

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When an animal was desired with skin in the late growing phase (late anagen) the animal was plucked and used 12 days later (if the above changes had been noted). For telogen skin animals were plucked and, if satisfactory were used 25 days later. For skin in the early growing phase (early anagen) hair was plucked and following the usual changes was replucked 25 days later (while in telogen). The skin was used 1 day after replucking that is 29 days after the original plucking. Any animal in which the skin phase could not be identified accurately was discarded for the purpose of the experiment. Before grafting all remaining hair on the dorsum was removed with a commercial depilatory.*

A single donor animal was used to donate skin grafts to several recipient animals. In the grafting technique used the skin graft had the panniculus carnosus removed and the recipient bed had the panniculus carnosus and its associated blood vessels intact (modified from Edgerton)¹¹

Donor skin was taken in each of the 3 phases and grafted to individual recipient beds in each of these phases. Control experiments were also done. These were autograft experiments (skin grafted from 1 animal to the same animal) to demonstrate the dependability of the method of grafting and isograft experiments (skin grafted from 1 individual of an inbred strain to another of the same strain) to demonstrate the genetic uniformity of the animals.

Each homograft experiment initially involved grafting skin from 1 Balb/c mouse to 4 C₅₇Bl/6 mice and from 1 C₅₇Bl/6 mouse to 1 Balb/c mice. Whenever experimental results were in doubt (due to lack of sufficient animals surviving) or where a check on the results was desired additional grafting experiments were done.

The donor animal was anesthetized by intraperitoneal Nembutal** (0.85 mg/10 gm body weight). The operative area was prepared with a pHiso hex*** and Zephiran**** scrub. Several donor sites (1.5 cm) were marked on the animal's dorsal skin using a template and methylene blue. Skin outside the marked area was then cut into with a small curved scissors. The scissors were used points up to create a cleavage plane between the panniculus carnosus and the fatty layer of the skin. The graft was cut along 3 sides and laid back. Any remaining panniculus was very carefully pulled away from the graft. Locke's solution was used to keep the tissues moist. The skin graft was put back in place while the recipient animal was prepared. When ready the fourth side was cut, the skin graft transferred to the recipient bed and the donor animal sacrificed.

The recipient animal was prepared in the same way with an area 1.5 cm square being marked on the dorsum using a template and methylene blue. The skin was then tented along 1 side of this area and a slight shallow cut made using small curved scissors. With traction on the cut skin edge in a direction opposite to the cutting of the scissors a narrow strip of skin was cut from the underlying panniculus carnosus around the 4 sides of the recipient site. The underlying panniculus carnosus and its associated blood vessels were kept moist with sterile Locke's solution. When the recipient site was

*Sur-Cutter Products Inc. NYC NY—The active ingredient is calcium thio-glycolate.
Nembutal (pentobarbital) Abbott Laboratories North Chicago, Ill.

**pHisoHex Winthrop Stearns Inc. NYC NY

***Zephiran Chloride Winthrop Stearns Inc. NYC NY

work the possible importance of the skin cycles of the rodent has been overlooked. The changes seen in the skin during a skin cycle are very marked and could well introduce errors into experimental results.

Skin cycles are a regular synchronized waxing and waning of practically all of the morphological and biochemical components of the skin.^{7-9,10,11} The skin cycle is divided into 3 phases. The first is a growing phase called *anagen* during which all hair growth in a given area takes place. This lasts about 18 days and is followed by a 2 day regressive phase called *catagen* and then by a 1 to 10 day resting phase called *telogen*.¹⁰ The growing phase has been divided into subphases on the basis of morphological changes in hair follicle development.⁴⁻⁶ Other factors such as the estrus cycle,¹² the environmental temperature,¹⁴ the daily activity,² food ingestion^{1,4} and minor trauma such as bites and scratches have been shown to cause appreciable changes in the day to day morphology and physiology of rodents' skin. In addition it would appear that genetic differences between males and females of the same strain affect the survival of skin grafts.

When hair is pulled out in a given area (by plucking or by bites, scratches and cuts) the onset of a new cycle is hastened. This induced cycle is limited to the area plucked and involves all the skin components. Cutting the hair without pulling it out appears to have no effect on the skin cycle.^{6,17} If the hair in a given area is plucked in telogen a new cycle begins almost immediately at the beginning of anagen. This makes it possible to predict and control the phase of the skin cycle.

During the first few naturally occurring cycles there is also some relationship between the age of the animal and the stage of the skin cycle on the animal's dorsum. However this is probably not a reliable way of determining the stage of the skin cycle.¹⁸

METHOD

C57Bl/6 and B6B1b/c male mice from the Roscoe B. Jackson Memorial Laboratory were used to insure a constant difference between donor and recipient. The mice were isolated in individual cages in a room kept at $70 \pm 1^\circ\text{F}$. The skin cycle was controlled by inducing a new cycle on adult animals (over 90 days old) whose skin appeared to be in telogen. The dorsal hair was plucked by hand, and then several coats of an epilating wax* were applied to remove all remaining visible hairs.

To be absolutely certain of the phase of the skin the plucked areas were inspected daily for the appearance of certain characteristic changes indicating the onset of anagen. These consist of a thickening of the skin by the 5th day, darkening of the skin in pigmented animals by the 7th day, and eruption of the hair shaft by the 8th to 9th day. If the plucking is erroneously done in some phase other than telogen these changes do not occur or they occur on a different time schedule.

Three phases of the skin cycle were chosen for investigation. The early growing phase (early anagen), the late growing phase (late anagen) and the resting phase (telogen). These phases were selected because each is markedly different from the others. In addition early anagen was selected because grossly it is sometimes difficult to distinguish from telogen.

*Zip epilator, Jordan Inc. NYC, NY. This when heated in a water bath to its melting point was found to be satisfactory.

C Bl/6 mice were used. The average survival time was 3.1 days. The endpoint occurred on the 3rd day in 5 grafts and on the 1th day in 3 grafts.

C Late anagen skin grafted to recipients in telogen. 1 Balb/c and 1 C Bl/6 mice were used. Two grafts were unsatisfactory and were discarded. The remaining 6 grafts had an average survival time of 3.0 days. All endpoints were on the 3rd day.

H Late anagen skin grafted to recipients in early anagen. 7 Balb/c and 8 C Bl/6 mice were used. Light grafts were unsatisfactory and were discarded. The remaining 7 grafts had an average survival time of 3.3 days. The endpoint occurred on the 3rd day in 5 grafts and on the 1th day in 2 grafts.

I Late anagen skin grafted to recipients in late anagen. 1 Balb/c and 5 C Bl/6 mice were used. Two grafts were unsatisfactory and were discarded. The remaining 7 grafts had an average survival time of 3.3 days. The endpoint occurred on the 3rd day in 5 grafts and on the 4th day in 2 grafts.

Table 1 The Survival Time of Skin Homografts Measured in Days Showing Variations Due to the Phase of the Skin Cycle of the Graft and the Recipient Bed

SKIN GRAFT	RECIPIENT BED		
	TELOGEN	EARLY ANAGEN	LATE ANAGEN
Telogen	7.5	5.1	5.2
Early Anagen	4.2	3.6	3.4
Late Anagen	3.0	3.3	3.3

SUMMARY AND CONCLUSION

These results indicate that telogen skin homografts survive as much as twice as long as anagen skin homografts and that they lasted longer than grafts in any other stage regardless of the phase of the cycle in the recipient bed. The phase of the recipient bed does seem to exert some effect on the duration of the skin homograft survival. This is most clearly demonstrated where telogen skin was grafted to anagen recipient beds. These figures are thought to be significant because in any given group all the homografts displayed an endpoint reaction within a space of 48 hr. at most. In fact the endpoint in any 1 group occurred within a space of 24 hr. in all but 3 mice of the 73 animals observed. These results tend to confirm the work of Ballantyne and Converse in rats.

It would seem that skin cycles exert an appreciable effect on the survival time of skin homografts in the mouse. Skin cycles should probably be controlled in skin homograft experiments on the mouse, the rat and the rabbit where the survival time of skin homografts is to be measured.

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completely cleared the donor skin was freed and adjusted in place, the excess skin being trimmed so that the graft was tailored to fit the recipient area. No sutures, glue, or dressing were used. The animal was kept anesthetized for several hours to permit the graft to agglutinate in place.

The endpoint consisted of observing a subgraft hemorrhagic reaction which is visible to the naked eye as small ecchymotic areas. This usually occurs first in isolated areas later spreading to include the entire graft. The skin overlying such hemorrhages becomes crusted and necrotic and eventually sloughs away. This hemorrhagic spot is remarkably abrupt in its onset and seems to be in a fairly constant relationship to the cessation of graft viability. It has been correlated by Edgerton¹¹ with the microscopic changes seen at the site of a homograft rejection reaction.

After grafting the recipient animals were inspected at the end of each 24 hr. interval to determine the condition of the graft. A few grafts displayed necrotic areas within 48 hr. possibly due to serious interference with the blood supply to the panniculus. The graft was considered unsatisfactory and was not included in the tabulation of results if it displayed extensive necrotic areas within 48 hr. if the animal died while still under anesthesia or if the animal was injured to remove the graft (always within the first 48 hr.).

RESULTS

Autograft Experiments Three Balb/c and 3 C₇Bl/6 mice received autografts. Survival of the autograft was assumed if it lasted for more than 30 days and a growth of hair was noted on the graft. All 6 autografts survived.

Isograft Experiments Twelve Balb/c and 7 C₇Bl/6 mice were isograft recipients. Six grafts were unsatisfactory and were discarded. Survival of the isograft was assumed if it lasted for more than 30 days and a growth of hair was noted on the graft. The remaining 11 isografts survived.

Homograft Experiments A T₁elogen skin grafted to recipients in telogen. 9 Balb/c and 8 C₇Bl/6 mice were used. Six grafts were unsatisfactory and were discarded. The remaining 11 grafts had an average survival time of 7.3 days. Endpoints occurred on the 6th day in 2 grafts and on the 7th day in 4 grafts and on the 8th day in 5 grafts.

B Telogen skin grafted to recipients in early anagen. 7 Balb/c and 8 C₇Bl/6 mice were used. One graft was unsatisfactory and was discarded. The remaining 11 grafts had an average survival time of 5.1 days. Endpoints occurred on the 4th day in 1 graft, on the 5th day in 10 grafts, and on the 6th day in 3 grafts.

C Telogen skin grafted to recipients in late anagen. 6 Balb/c and 8 C₇Bl/6 mice were used. Five grafts were unsatisfactory and were discarded. The average survival time of the remaining 9 grafts was 5.2 days. Endpoints occurred on the 5th day in 7 grafts and on the 6th day in 2 grafts.

D Early anagen skin grafted to recipients in telogen. 1 Balb/c and 1 C₇Bl/6 mice were used. Two grafts were unsatisfactory and were discarded. The remaining 6 grafts had an average survival time of 4.2 days. The endpoint occurred on the 4th day in 5 grafts and on the 5th day in 1 graft.

E Early anagen skin grafted to recipients in early anagen. 6 Balb/c and 1 C₇Bl/6 mice were used. The average survival time was 3.6 days. The endpoint occurred on the 3rd day in 1 graft and on the 4th day in 6 grafts.

F Early anagen skin grafted to recipients in late anagen. 1 Balb/c and 1

Approximately six hundred 2 to 3 mm square human skin explants from 31 patients were studied. These were taken as thin split thickness grafts in the operating or autopsy room. The skin was preserved from 1 to 70 days at 1°C in 10 ml of a nutrient media consisting of 10 per cent human placental cord serum, 90 per cent Simm's γ balanced salt solution and 100 units of penicillin/ml.

Two tissue culture methods were utilized: the Maximow lying drop double cover slip and Correl D5 flasks. The supporting clot for both methods consisted of 1 part chick plasma (Disco), 1 part 9 day chick embryo extract with Simm's γ , 1 part human placental cord serum and 100 units of penicillin/ml. The liquid nutrient consisted of 2 parts human placental cord serum, 1 part Simm's γ , 1 part 9 day chick embryo extract and 100 units penicillin/ml. The liquid nutrient was added to the supporting clot every 3 days beginning on the third day with the lying drop and on the first day with the Correl D5 flasks.

Results indicated adequate growth of fibroblasts and epidermal elements often; however, after a long period of some days for the thicker skin explants. Ordinary operating room skin preps failed to insure the rigid sterility required for tissue culture. Contamination could be routinely prevented only if an antibiotic in proper concentration were added. Also certain skin preparation agents cause a chemical toxicity to sterile tissue cultures.

Viable cultures were obtained from living patients up to 21 days after preservation at 4°C in the 10 per cent serum nutrient media. However, longer preservation times decreased the chances for a viable culture as indicated in the tabular data. Viable cultures were obtained from postmortem skin taken up to 10 hr after death and preserved at 4°C in the 10 per cent serum nutrient media up to 11 days.

Criteria of viability for preserved human skin grafts is a difficult subject but many workers feel that tissue culture methods offer the best qualitative

Table 1 Tissue Culture Results of Human Skin Grafts

STORAGE TIME	NUMBER OF PATIENTS	GROWTH RESULTS
1-3 days	8	8 Good
4-8 days	4	2 Good 1 None 1 Contaminated
9-14 days	4	2 Good 2 None
16-28 days	4	1 Good 2 None 1 Contaminated
29-70 days	5	None

Table 2 Tissue Culture Results of Postmortem Human Skin Grafts

TIME AFTER DEATH	STORAGE TIME	GROWTH RESULTS
2 hr	11 days	Good
4 hr	8 days	Good
6 hr	1 day	Good
8 hr	5 days	Good
9 hr	6 days	Good
10 hr	1 day	Good

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VIABILITY STUDIES OF HUMAN SKIN CRAFTS WITH TISSUE CULTURE METHODS*

ROBERT M. McCORMACK

Numerous human tissues preserved at low temperatures may be clinically useful as transplanted tissues without the exacting criteria of viability of the preserved cells. However when skin is required is a transplanted tissue the transplanted cells must remain viable to be permanently clinically useful. The permanent replacement of viable epidermis for a full thickness skin loss of large size can be obtained only from a viable skin autograft (fresh or preserved). The clinical necessity for viable human skin autografts points to the need for further laboratory information on the criteria and conditions to insure this viability. As Allgower and Blocker¹ point out tissue culture studies are very exciting tests for survival studies of stored skin. Tissue culture is generally considered a more stringent test than reimplantation on the donor or performing a heterograft to a laboratory animal.

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With the technical assistance of Miss Evelyn Peters

THE DIFFERENTIAL STAINING OF THE PAROTID GLAND AS A PRELIMINARY TO PAROTID SURGERY*

HERBERT J. TORREST

A project was set up with the specific aim of finding a method of staining the parotid gland in the live animal without staining the surrounding tissue or facial nerve. The anatomy of the parotid gland, Stensen's duct and facial nerve of the dog was studied. This was done by dissecting dog cadavers. It was found that the relationship between the facial nerve and the parotid gland was similar to man's except in the dog the branches of the facial nerve were relatively larger and the parotid gland lay almost entirely superficial to the nerve. Usually a small projection of the parotid gland extended down between the 2 main branches of the facial nerve. The parotid duct was found to be small and fragile and sometimes difficult to identify.

METHOD

The experimental work was done using live dogs under anesthesia. Aseptic operative technique was used. In each case Stensen's duct was exposed as it crossed the masseter muscle. A small needle was inserted into the duct and $\frac{1}{2}$ to 3 cc. of dye was injected into the gland. Only relatively nontoxic dyes were tried e.g. indigo carmine, Evans blue and methylene blue. Parotidectomies were done at varying intervals following the injection. Twenty-four operative procedures have been done to date.

RESULTS

It was found that in the live dog the parotid gland could be stained precisely and completely without staining the surrounding tissue or facial nerve. With the gland exposed it was seen to distend and take on the color of the dye. The gland capsule was an effective barrier and contained the dye. The facial nerve remained white and stood against the color of the stained gland.

Evans blue in 15 per cent solution injected into the parotid duct system stained the gland very well. However it had the disadvantage of being picked up by the lymphatic system. If immediate parotidectomy was not done the surrounding face and neck subcutaneous tissue would take on a light blue color.

Indigo carmine in 8 per cent solution stained the parotid gland adequately but was less bright than Evans blue or methylene blue. Methylene blue in 1 per cent solution was studied most because it seemed to do the best job of staining the gland. Microscopic study of methylene blue stained parotid tissue was done using frozen and paraffin imbedded sections. It was believed that the methylene blue entered *only* the ramifications of the parotid duct system. No staining or damage to the gland cells could be seen.

DISCUSSION

It was found that there was no advantage in waiting for the gland to take up the stain. Immediate parotidectomy could be done without any danger of dye leakage even if the gland tissue was invaded. In the dog the parotid

*From the Department of Plastic Surgery, University of Kansas Medical Center, Kansas City, Kansas.

evidence of intact cellular function under the most rigid controls. Many criteria have been utilized for example, the gross and microscopic appearance of human skin grafts on the chorio-allantoic membrane of chick embryos (Tenery and McDowell²) and on autotransplantation to granulating surfaces (Allgower and Blocker,¹ Georgiade *et al*,³ and Brown *et al*,⁴) biochemical criteria such as oxygen uptake utilized by Skoog and Georgiade *et al*,⁵ and enzymatic activity by Charnness *et al*.⁶ Heterografts from human to the mouse have been used for large scale testing (Brown *et al*,⁷) by gross appearance.

Relatively standard tissue culture techniques with available commercial media can make such techniques practical for the evaluation of viability of preserved human skin grafts. Such a laboratory check should be used on preserved postmortem homografts as there is a considerable clinical difference between use as a viable temporary homograft that takes and a non-viable dressing material that fails to take, necrotizes within a few days and may postpone the time when permanent healing with autografts can be accomplished.

SUMMARY

1. Tissue culture of human skin grafts particularly after storage is an exacting qualitative test of viability.

2. Viable skin tissue cultures were obtained up to 3 wks after storage in a 10 per cent serum nutrient medium at 1°C.

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gland appeared to blend with the surrounding areolar tissue. After being stained it was outlined precisely and could be dissected readily. Finally separation of the facial nerve from parotid tissue was made much easier.

The only complication noted was some edema of the adjacent subcutaneous tissue if the stained gland was left in place. This was found if the face was reopened 1 to 4 days after the dye injection. The surgical wound healed well in all cases after the parotidectomy. Also the surgical wound healed well if the stained parotid gland was left *in situ*.

Preliminary clinical trial has been done. This has shown that the human parotid can be stained with an intra-ductal injected dye. Results of clinical use of this method of staining the parotid gland will be detailed in a later paper.

SUMMARY

The parotid gland of the dog can be stained by injecting 1.5 to 3 cc of an appropriate dye into Stensen's duct. No other tissue outside the gland is stained. Parotidectomy and separation of the facial nerve from the gland is greatly facilitated by preliminary staining of the parotid gland. It is hoped this method of dyeing the parotid gland can be utilized to simplify human parotid surgery.

A STUDY OF THE PATHOLOGIC ANATOMY OF THE PROXIMAL TUBULES OF THE MOUSE PRODUCED BY VARIOUS NEPHROTOXINS*

C. BARBER MULLER AND A. D. MASON, JR.

The pathologic anatomy of renal tubular lesions is less well understood than the pathologic physiology of acute renal failure which has been of great interest in recent years. The clinical course of patients suffering from acute renal failure following the ingestion of nephrotoxins is somewhat different and seemingly less fulminant than that of acute renal failure which follows crushing or wounding and although extrarenal factors may well be responsible for this apparent dissimilarity it is possible that differences in tubular pathology may be the cause. In a previous communication¹ electron micrographs of normal dog kidneys and of dogs subjected to repeated transfusions of human blood were presented. The refined techniques of electron microscopy which are now currently in use have already out dated this previous study but some information of a comparative nature may be drawn between this present work and the prior report.

This study is a preliminary description of specific changes in the proximal tubule cells of mouse kidneys following the administration of mercuric chloride, uranyl nitrate and potassium dichromate.

METHOD

White female mice weighing 25 to 32 gm. and between 100 and 120 days old were used. In the mercury bichloride experiments 1.0 mg. of HgCl_2 in 1.0 ml. of water was injected subcutaneously and animals were sacrificed at 2, 4, and 6 hr. We had previously determined that 1.0 mg. HgCl_2 killed mice in 36 to 72 hr. No animal survived the injection of 1.5 mg. more than 3 hr. and 2 mice given 0.25 mg. survived for 3 wks. When animals were sacrificed 3 hr. after the administration of 1.0 mg. HgCl_2 the kidneys were usually pink white but by 4 to 6 hr. they had become pale though not particularly swollen. The mice did not appear particularly ill by 6 hours.

In another group of mice 1.0 mg. of uranyl nitrate was administered subcutaneously and in a third group 2.5 mg. of potassium dichromate was injected intraperitoneally. These animals were handled in a fashion similar to those in the mercuric chloride group.

Portions of the kidneys were fixed in 10 per cent formalin while specimens for electron microscopy were cut into 1 mm. cubes and dropped into Dalton's osmic acid fixative. After dehydration in alcohol the specimens were embedded in methacrylate, sectioned with a glass knife and examined in an RCA LMU electron microscope.

*From the Department of Surgery, Washington University, St. Louis, Mo. Supported by U.S. H.S. Grant A 96 (C4).

RESULTS

Routine formalin fixed sections embedded in paraffin and stained with hematoxylin and eosin, do not show any necrosis or sloughing of the tubules at this time.

After mercuric chloride and uranyl nitrate injections examination of proximal distal and collecting tubules with the electron microscope confirmed the light microscope impression that there was no gross destruction of cellular integrity. However all cells showed a progressive dissolution of the mitochondria. There seemed to be fewer deranged mitochondria in the pink kidneys of the mice sacrificed at 3 hr than of the pale kidneys seen at 6 hr but a statistical evaluation is not possible with such small samples. All cells particularly in those of the proximal convoluted tubules contained all stages of mitochondrial dissolution from normal intact mitochondria to pale washed out disrupted ghosts. The pale 6 hr kidneys contained mostly ghosts the pink 3 hr kidneys mostly well formed mitochondria but in both groups all stages were recognizable. Basement membranes cell borders brush borders and the background cytoplasm of the cells appeared intact.

The normal mouse mitochondrion has been well described and pictures of it are not presented here. There is a double membrane around the periphery and transverse cristae which are also comprised of a double membrane. Each single membrane is approximately 50 \AA in thickness and the space between them 70 \AA .^{2,3} The mitochondrial material itself is much more electron dense than the background cytoplasm of the cell and is generally homogeneous throughout the mitochondrion except for a dense spherical body 800 to 500 \AA , which is seen in most mitochondria.

Figure 1 is a low power view of a proximal tubule cell of a mouse poisoned with mercuric chloride. In the lower portion is the basement membrane at the top are under portions of the brush border. In the central portion are three fairly normal mitochondria (a). Disruption of the peripheral membranes can be seen at (b) while cristae remain intact. Vacuolization of the

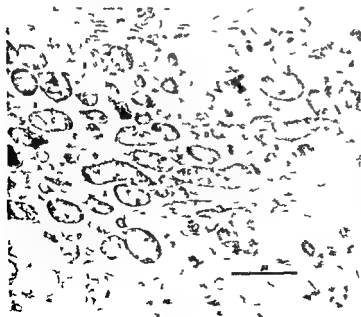


Fig 1 Mitochondrial dissolution 3 hr following mercuric chloride (For explanation of figures see text)

Fig. 2 Mitochondrial dissolution following mercuric chloride



mitochondrion with progressive loss of cristae is seen at (c) and the hollow disrupted completely vacuolated mitochondrial ghost at (d)

Figure 2 shows several phases of the early peripheral membrane disruption (b) and vacuolization (c). This kind of degenerative changes seems to be the most common. Poisoning with uranyl nitrate gives lesions similar in all respects to those seen with mercuric chloride.

Figure 3 shows at high magnification the loss of peripheral membrane (b) and disruption (c) of the internal structure.



Fig. 3 Mitochondrial dissolution following mercuric chloride

RESULTS

Routine formalin fixed sections, embedded in paraffin and stained with hematoxylin and eosin, do not show any necrosis or sloughing of the tubules at this time.

After mercuric chloride and uranyl nitrate injections, examination of proximal, distal and collecting tubules with the electron microscope confirmed the light microscope impression that there was no gross destruction of cellular integrity. However, all cells showed a progressive dissolution of the mitochondria. There seemed to be fewer deranged mitochondria in the pink kidneys of the mice sacrificed at 3 hr than of the pale kidneys seen at 6 hr but a statistical evaluation is not possible with such small samples. All cells particularly in those of the proximal convoluted tubules contained all stages of mitochondrial dissolution from normal intact mitochondria to pale washed-out disrupted ghosts. The pale 6 hr kidneys contained mostly ghosts the pink 3 hr kidneys mostly well formed mitochondria but in both groups all stages were recognizable. Basement membranes cell borders brush borders and the background cytoplasm of the cells appeared intact.

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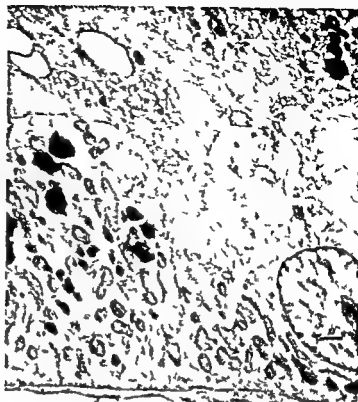


Fig 4 Cellular changes following administration of potassium dichromate

Figure 4 shows the pale cytoplasm shrunken condensed mitochondria and crisp fixed cell membranes which are present following the administration of potassium dichromate

DISCUSSION

Simonds and Hepler¹ have presented evidence that mercuric chloride and uranyl nitrate damage the distal portion of the proximal tubule while potassium dichromate has a predilection for its more proximal portions. The work presented here indicates that uranyl nitrate and mercuric chloride attack a similar portion of the tubule and produce similar effects within the cell while potassium dichromate creates an entirely different lesion both as to locus in the tubule and type of degeneration in the cell. All 3 of these nephrotoxins produce renal tubule disease which in its pathologic anatomy appears to be different from that caused by the infusions of mismatched blood into dogs.¹

CONCLUSIONS

Mercuric chloride and uranyl nitrate cause renal tubular necrosis that starts with dissolution and vacuolization of the mitochondrion.

Potassium dichromate causes renal tubular necrosis that commences with mitochondrial coalescence and washed out appearance in the background cytoplasm and alterations of the cell membranes.

Electron microscope pictures visualizing these changes are presented.

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MECHANISMS OF HYDRONEPHROSIS THE ROUTE OF BACKFLOW*

LESTER PERSKY, FREDERICK J BONTÉ AND CHARLES A HUBB

The initial stimulatory studies by Hinman¹ concerning the nature of hydro-nephrosis demonstrated that pelvic contents in cases of obstruction gain access to the blood stream. A variety of substances²⁻³ varying from dyes to bacteria have been utilized since then in the pursuit of the exact route of this backflow. The development of radioactive isotopes and exact counting techniques suggested that the use of these substances would afford a more accurate technique for a study of hydronephrosis and a more definitive tracing of the reflux. In previous communications⁴⁻⁵ we showed that particle size did not mitigate against backflow, that the backflow occurred rapidly in the acutely obstructed kidney and by means of radioautographs we demonstrated that this access to the vascular tree appeared to be a combination of pyelotubular and pyelointerstitial routes.

The recent work of Goodwin⁶ coupled with contributions from the foreign literature⁷⁻⁸ focused our attention upon the renal lymphatics. This study was undertaken in order to prove or disprove the role of these channels in hydronephrosis.

METHOD

All experiments were carried out on healthy mongrel dogs weighing from 10 to 14 kg. Nembutal anesthesia was employed routinely. After exposing the kidney complete obstruction was created by ligating the ureter close to the ureteropelvic junction about a special 3 way needle leading to a mercury manometer and through which radioactive isotopes could be instilled into the pelvis without modifying intrapelvic pressures. This was done at regular 5 min intervals throughout the course of each experiment to permit and insure complete mixing of urine and the radioactive substance. Two hun-

*From the Department of Surgery, Urological Service and the Department of Radiology, Western Reserve University and the University Hospitals, Cleveland, Ohio. Supported in part by the Beaumont Fund, Cleveland, Ohio and conducted in part with facilities made available through contract number W-31-109-ENG-78 between the United States Atomic Energy Commission and Western Reserve University.

dred and fifty microcuries of iodinated albumin (I^{131}) (molecular weight approximately 60 000) were used for each animal. This substance was utilized because of the large particle size which eliminated the possibility of simple diffusion and absorption.

The animals were divided into 3 experimental groups. In Group 1 the kidney was completely mobilized from all perirenal fat and the hilar vessels completely stripped of their surrounding fat, areolar tissue, nerves and presumably lymphatics. After obstructing the ureter and after the intermingling of the pelvic contents with iodinated albumin as previously described, blood samples were drawn at 5 min intervals for 1 hr.

In Group 2 the thoracic duct was cannulated with a small polyethylene catheter close to its entrance at the junction of the subclavian and jugular veins in the neck. The ureter was again ligated about the 3 way needle disturbing the kidney as little as possible and blood and lymph collected at 5 min intervals for 1 hr. In Group 3 a similar experiment was carried out except that the kidney was once more completely mobilized and the renal vessels stripped. Four animals were employed in Groups 1 and 2 and 2 animals in Group 3.

RESULTS

Consistent results were obtained in all animals within each group. In Group 1 where the kidney and vessels were stripped, radioactivity in the blood stayed at low levels but gradually rose slightly at the end of the experimental period of observation. A typical animal of this group is seen in Figure 1. In Group 2 in which the kidney was undisturbed and the thoracic duct cannulated, the radioactivity in the blood stream was of the same order of magnitude as was noted in Group 1 but the counts obtained from the thoracic duct lymph were elevated to a much greater degree (Fig 2). In Group 3 (Fig 3) with the kidney stripped again and a cannula in the thoracic duct we once again have the same general level of counts obtained in the peripheral blood but no or little radioactivity detected in the lymph. The scale of these figures although not exactly the same has been modified to permit representation of the counts and does not distort the findings. If exactly parallel scales had been used the blood levels in Groups 1 and 3 would have been barely discernible.

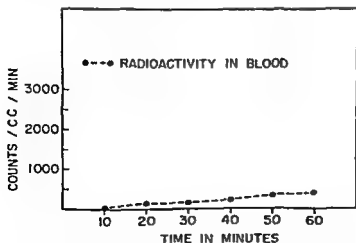
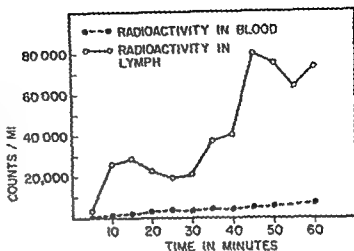


Fig 1 Representative curve of radioactivity detected in the blood stream following the installation of iodinated albumin in the obstructed renal pelvis of the completely mobilized kidney.

Fig 2 Representative curves of radioactivity detected in the blood and thoracic duct lymph following the installation of iodinated albumin in the obstructed pelvis of an undisturbed kidney



DISCUSSION

These studies corroborate the contention of Goodwin and others that the renal lymphatics play an important role in the mechanism of hydronephrosis. The counts in the thoracic duct lymph exceed by far any of the previously observed levels in our initial studies (Fig 4) wherein radioactivity was noted in the peripheral blood following the installation of iodinated albumin, ionic I^{131} and colloidal gold. The level of radioactivity in the blood detected following the use of colloidal gold is misleading due to the rapidity of the reticuloendothelial system in clearing it from the blood stream. It is presumed that in the present experiments in which the kidney was completely mobilized and the vessels denuded that the lymphatics were divided completely. The ultimate appearance of the iodinated albumin in the blood stream in all groups may represent reflux into capillaries secondary to pyclo interstitial backflow as suggested by Fuchs⁹ and Narath¹⁰ or may be a reflection of a few lymphatics which were undivided and had alternative entrances into the blood stream.

The pattern of the curves of radioactivity found for the thoracic duct lymph in Group 2 is analogous to the curves found previously in the blood when the duct has not been cannulated and the kidney not disturbed. The peak corresponds to the period after the fall in pelvic pressure and which

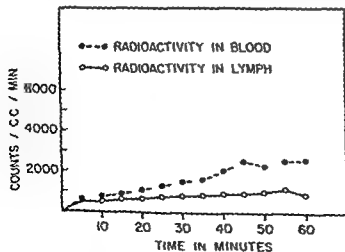


Fig 3 Radioactivity in the blood and lymph after completely mobilizing the kidney

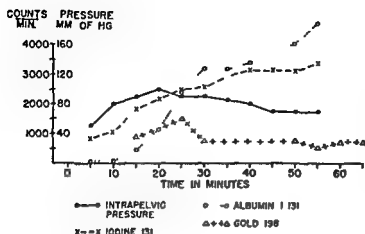


Fig 4 Representative curves for the backflow obtained utilizing iodinated albumin I^{131} and colloidal gold

usually heralds the onset of backflow. It is probably coincidental to the escape of pelvic fluid via the tubules or interstitial spaces into the lymphatics.⁴ It is hoped ultimately to reduplicate these experiments in more chronic states of obstruction and in situations where the perirenal lymphatics have been modified by inflammatory or sclerosing agents. If no backflow is obtained under these conditions or under conditions of auto or homotransplantation, then the evidence will be almost complete for the thesis to which we now subscribe, namely that the renal lymphatics are the main route of backflow in cases of obstruction.

SUMMARY

In a series of experiments utilizing iodinated albumin in the obstructed kidney of the dog by cannulation of the thoracic duct the renal lymphatics were demonstrated to be the main route of backflow. When the lymphatics were divided, radioactivity in the lymph fell to low levels further supporting this concept.

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HYDRODYNAMICS OF THE HUMAN URETER AND RENAL PELVIS*

WILLIAM H. RATTNER, SIDNEY LINK AND JOHN J. MURPHY

The problems of hydronephrosis, hydroureter, ureterovesical reflux, etc., confront the urologist frequently. The solution to these problems on a physiologic basis requires an understanding of the normal hydrodynamics in the upper urinary tract. While considerable knowledge has been gained from recording bladder pressures and activity, relatively little is known about the basic pressure phenomena of the renal pelvis and ureter. This study was done to record the basal pressure and peristaltic activity of the intact human ureter and renal pelvis.

METHOD

Seventeen adult females with normally functioning urinary tracts were studied. Women were chosen because of the ease with which urethroscopy could be carried out and a ureteral catheter could be held securely. The subjects all had a recent normal intravenous urogram, and a normal blood urea nitrogen determination. A #4 F ureteral catheter was passed to the renal pelvis and a #20 F catheter inserted into the bladder. The catheters were connected to Statham P23B electrical transducers. The resulting current was amplified and recorded by means of a Sanborn Poly Viso Recorder equipped with strain gauge amplifiers. A simultaneous chest pneumograph was taken. After a suitable period of recording from the renal pelvis the ureteral catheter was withdrawn 7 to 9 cm. so that its tip was located in the midureter and another tracing made. The catheter was then withdrawn an additional 8 to 10 cm. to the lower third of the ureter, and the recording continued. The location of the tip of the ureteral catheter was established by a plain film of the abdomen on several occasions. Patency of the recording system was assured by noting pressure changes caused by lifting the head, straining and deep breathing. The patients received no premedication or anesthesia. The volume of urine excreted during the test was measured and its pH recorded.

RESULTS

Pressure and Amplitudes. Definite periodic pressure changes were observed on records from the renal pelvis, mid and lower thirds of the ureter in all cases. The absolute pressure values are recorded in Figure 1. All values (basal pressure and amplitude) are measured as centimeters of water pressure. The basal pressure in the renal pelvis varied from 6 to 25 cm. with an average of 14.7. The average amplitude of waves in the renal pelvis was 4.1 cm. Similar amplitudes were noted in the mid ureter although the basal pressure was slightly higher (17 cm.). The basal pressure in the lower third of the ureter was 14.3 cm. but the amplitude of the periodic pressure changes was significantly higher. They averaged 8.9 cm. of water pressure.

Pattern of Waves. At least 4 distinct wave patterns were encountered. These were the monophasic, diphasic, triphasic and a slow undulating wave.

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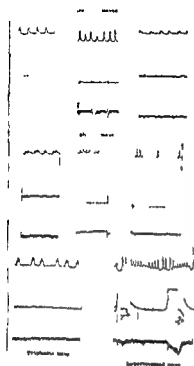


Fig 1 The appearance of the phasic waves in the renal pelvis and ureter is recorded through an open lumen catheter. Simultaneous ureteral (top) bladder (middle) and pneumographic (lower) pressure recordings. Amplitude: One mm equal to 1 cm of water pressure except where indicated in the lower right record. Speed: 0.25 mm/sec (3 large squares/min) except in the upper and lower left records where 6 large squares represent 1 min.

upon which other types of waves were superimposed (Fig 1). The latter occurred only once in the study, in association with a voiding contraction of the bladder. The other wave patterns were observed in each of the 3 areas studied. Different types of waves occurred at the same level, and in the same patient. While the waves usually had a definite rhythm, it was not unusual to find isolated bursts of 2 (couplets) or 3 (triplets) (Fig 2). One patient was given 1 lorine (tricyclamol chloride) in a dose sufficient to cause verostoma, but this drug did not in any way influence the ureteral activity in this patient. One patient with megaloureter was studied and found to have renal and ureteral pressure recordings similar to those from the normal ureter and renal pelvis. The urine volume and pH did not appear to have any correlation with the peristaltic activity in this study.

DISCUSSION

Several methods have been used in the study of renal pelvic and ureteral motor physiology.¹ These include direct observation of the isolated and intact ureter, cystoscopic evaluation of the size and force of ureteral peristalsis,²⁻³ direct observation of the exteriorized trigone,⁴ measurement of the action potentials of isolated and intact ureteral segments,⁵⁻⁶⁻⁷⁻⁸ cinefluorography,⁹ and pressure studies using the hydrophorometer or one of its modifications.¹⁰⁻¹¹⁻¹²⁻¹³⁻¹⁴ None of these techniques recorded absolute pressure values, and most of them required subjective interpretation of the measurements. In addition, the introduction of a balloon or large cannula produces an abnormal pattern of peristalsis in any hollow viscus. The authors feel that the use of a fine ureteral catheter connected to a sensitive external recording device produces the least possible constraint artifact in a study of this kind. A similar technique has been used recently in cardiac and gastrointestinal pressure studies.¹⁵⁻¹⁶ The basal pressures in all 3 segments studied were relatively constant, but it is interesting to note that the lower third of the ureter

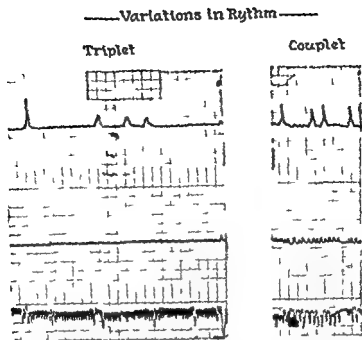


Fig 2 Simultaneous ureteral (top) bladder (middle) and pneumographic (bottom) tracings showing 3 waves occurring together (triplet) and a two wave sequence (couplet). Amplitude 1 mm represents 1 cm of water pressure. Time: On the left 0.2 mm/sec (3 large squares/min) On the right 0.5 mm/sec (6 large squares/min)

had the most vigorous peristaltic activity. This may explain why reflux is so common after reimplantation of the ureter. If in addition to ablating the natural valve at the ureterovesical junction, one also removes the most active peristaltic segment, reflux would seem almost inevitable. The isolated observation on one patient who was given an anticholinergic drug would tend to lend support to the earlier observations of Lapides, although more determinations are necessary in this field. This technique should be of value in studying the pressure relationships in hydronephrosis and other malfunctions of the renal pelvis as well as the problems of ureteral reflux, megalo-ureter, the effect of transplantation on the ureter, and the influence of drugs on the motor activity of the urinary tract.

SUMMARY

1. The basal pressure and peristaltic activity of the renal pelvis and ureter were studied in 17 normal adult females by means of an indwelling ureteral catheter connected to an external electrical transducer and strain gauge amplifier.

2. Four distinct wave patterns were found. These were the monophasic, diphasic, triphasic, and slow undulating wave upon which other waves were superimposed.

3. The average amplitude of the peristaltic waves was highest in the lower third of the ureter.

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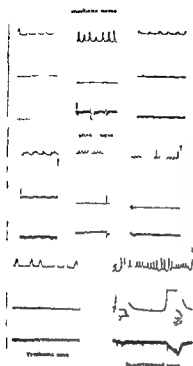


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






of the fluid bathing the ureteral segment was correlated with the action potentials recorded from the electrodes positioned in the ureter contained in the peritoneal recess. The lag of temperature change in the ureteral wall compared to that of the fluid about it, was measured in 2 experiments by using a needle thermocouple placed in the ureteral muscle. The effects of drugs upon ureteral activity were studied by placing drug solutions in peritoneal recesses by intravenous administration, and by injection into the ureteral wall. Conduction of waves in ureters having healed anastomoses also was investigated.²

RESULTS

Normal Peristaltic Action Potentials The action potential of a single peristaltic wave consisted of a spike followed by partial repolarization, a plateau and completion of repolarization. When the peristaltic wave originated in the ureter away from an electrode its action potential possessed an approach artefact (Table 1). When a wave arose at an electrode, no approach artefact was seen. That a peristaltic wave arose at a given electrode was also evidenced by its simultaneous appearance at equidistant electrodes above and below the electrode of origin. In most such waves, a slow rise of negativity (prepotential) was recorded from the electrode at the site of wave origin before the onset of the action potential spike (Table 1-4).

Post peristaltic Interval of Reduced Wave Velocity If 1 wave follows

Table 1 A Summary of Action Potential Shapes

TISSUE	POSITION OF THE ELECTRODE	TEMPERATURE	WAVE INTERVAL	RECORDED ACTION POTENTIAL
1 Ureter	Intraluminal away from site of wave origin	37° C	5 plus seconds	
2 Ureter	As in (1)	37° C	1 second	
3 Ureter	As in (1)	28° C	5 plus seconds	
4 Ureter	Intraluminal at site of wave origin	37° C	5 plus seconds	
5 Ureter	1 cm above the healed anastomosis	37° C	5 plus seconds	
6 Ureter	Intraluminal near the healed anastomosis	37° C	5 plus seconds	
7 Cardiac	Int. acellular pacemaker cells	37° C	0.6 seconds	
8 Ureter	Postulated true shape of the action potential at 37° C after a wave interval of 5 or more seconds			

Canine ureteral action potentials are shown in lines 1 to 6 inclusive. Line 7 shows the presence of a prepotential recorded intracellularly from pacemaker cells of cardiac muscle. Line 8 outlines the postulated true shape of the ureteral action potential. Whether or not the prepotential persists in an action potential conducted beyond its site of origin is as yet unknown.

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VARIATIONS IN THE SHAPE OF PERISTALTIC ACTION POTENTIALS OF THE CANINE URETER*

HARVEY R. BUTCHER JR. AND WILLIAM SLEATOR JR.

Despite many investigations of ureteral peristalsis the mechanism of its propagation along the ureter remains poorly understood. Consequently, a study of canine ureteral action potentials and the factors affecting their shape was undertaken to define accurately the electrical properties of the peristaltic conduction mechanism in an attempt to learn more concerning the transmission of the contraction wave through ureteral smooth muscle.

METHODS

Adult mongrel dogs of both sexes were anesthetized by intravenous injection of sodium pentobarbital (0.03 gm/kg). A cystostomy permitted easy intraureteral placement of an electrode assembly through the ureterovesical orifice. A multi-channelled modified Osner dynograph recorded the action potentials of peristaltic waves from the intraluminal electrodes of fine silver rubber (0.01" in diameter)^{1,2}. Waves were stimulated by distension of a small rubber balloon inserted into the upper ureter.

The effect of different temperatures upon ureteral conduction was studied by placing mammalian Ringer's solutions of varying temperatures into a peritoneal recess constructed about a segment of the ureter. Temperature

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






of the fluid bathing the ureteral segment was correlated with the action potentials recorded from the electrodes positioned in the ureter contained in the peritoneal recess. The log of temperature change in the ureteral wall compared to that of the fluid about it was measured in 2 experiments by using a needle thermocouple placed in the ureteral muscle. The effects of drugs upon ureteral activity were studied by placing drug solutions in peritoneal recesses by intravenous administration, and by injection into the ureteral wall. Conduction of waves in ureters having healed anastomoses also was investigated.²

RESULTS

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3 Ureter	As in (1)	28° C	5 plus seconds	
4 Ureter	Intraluminal at site of wave origin	37° C	5 plus seconds	
5 Ureter	In lumen of anastomosis	37° C	5 plus seconds	
6 Ureter	Intraluminal near healed anastomosis	37° C	5 plus seconds	
7 Cardiac	Intracellular Pacemaker cells	37° C	0.6 seconds	
8 Ureter	Postulated true shape of the action potential in interval of 5 seconds at 37° C after wave			

Canine ureteral action potentials are shown in lines 1 to 6 inclusive. Line 7 shows the presence of a prepotential recorded intracellularly from pacemaker cells of cardiac muscle. Line 8 outlines the postulated true shape of the ureteral action potential. Whether or not the prepotential persists in an action potential conducted beyond its site of origin is as yet unknown.

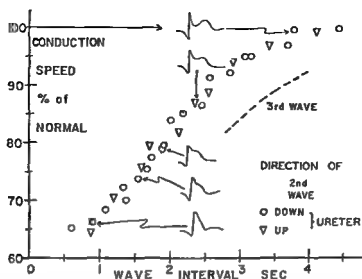


Fig 1 The Effect of Wave Interval The reduction in velocity and the change in action potential repolarization accompanying interperistaltic wave intervals of 3 sec or less is shown. These changes were unaffected by the direction of propagation of the second wave along the ureter. The dotted line labelled third wave shows the further reduction in velocity that occurs in a third wave stimulated at intervals of 4 sec or less after the second one.

another within 3 to 11 sec, the speed of the second wave is less than the first.¹ During the interval of reduced velocity the voltage time contour of the action potential of the peristaltic wave is altered (Fig 1). In the earlier part of this period that is just after passage of the distention refractory period (0.5 to 0.6 sec after the peristaltic wave) there is a reduction in the total duration of the action potential, a broadening of the negative peak phase with little change in voltage and no separation of repolarization into 2 phases by a plateau. Previous investigation¹ has correlated the degree of these action potential and velocity changes with a corresponding lessening of the strength of ureteral contraction.

The Effect of Different Temperatures Upon Peristaltic Action Potentials
Lowering of the temperature of an ureteral segment with cold mammalian Ringer's solution decreased the velocity of conduction, lowered the voltage of the plateau of the action potential and transformed it into oscillations (Fig 2). When the temperature of the solution immediately adjacent to the segment of ureter reached 22° to 24° C conduction through it ceased. At temperatures just above that required to produce block the velocity was the least and the plateau frequently not observed.

Elevation of the temperature of the ureteral environment above 37° C shortened the duration of the plateau, slightly increased the conduction velocity and increased the spontaneous activity of the ureter. Beyond 41° C velocity fell with temperature increase and finally at 45° to 46° C temperatures were reached which produced conduction block. A return of temperature to within physiologic limits was followed by a return of conduction if heating beyond the point of block had not been excessive or prolonged.

The Changes in Action Potential Shapes Associated with Healed Ureteral Anastomoses
Analysis of the effect of ureteral transection and anastomosis

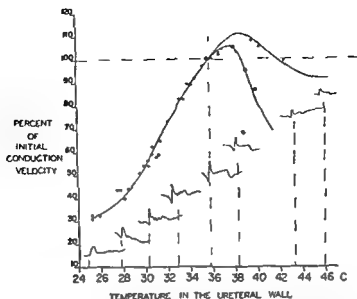


Fig 2 The Effect of Temperature Change Upon Ureteral Peristalsis The diagram shows the effect of various temperatures of the ureteral wall upon wave velocity and action potential shape The lower line of the velocity plot between 42 and 36 indicates the reduced wave velocities accompanying a return of conduction with cooling after conduction block had occurred at higher temperatures A decrease in voltage and a broadening of the peak phase of the action potential accompanied these waves

upon subsequent conduction of peristalsis and its relation to any associated hydronephrosis has been presented.² The action potentials conducted through the anastomoses and recorded from electrodes placed in the anastomotic lumen showed arrival of the action potential with an approach artefact in isoelectric period corresponding to the duration of delay in action potential conduction and a departure spike preceded by a pre potential and followed by an abnormal period of repolarization (Table 1 5). The latter consisted of marked oscillations or secondary spikes in place of the plateau of the action potentials. These plateau abnormalities were recorded in or adjacent to the ureteral scar in most instances and in all parts of a right ureter studied 2 mo after right nephrectomy ureteral transection and anastomosis (Table 1 6).

The Effect of Drugs Upon Action Potential Shape Nicotine curare hexa methonium xylocaine acetylcholine with and without eserine atropine epinephrine and regitine produced no change in velocity of conduction or shapes of the peristaltic action potentials. Histamine locally or systemically administered consistently increased the frequency of peristalsis but did not alter significantly the shape of the resulting action potentials. Its effect upon conduction velocity consisted of a shortening of the period of reduced wave velocity. In other words during the high frequency peristalsis caused by histamine the decrease of velocity caused by the recent passage of a preceding wave was less than normal. The administration of tripeleminamine citrate (Pyribenzamine) or diphenhydramine hydrochloride (Benadryl) decreased the voltage and broadened the peak phase of both the action

potentials of histamine stimulation and those initiated by distention until they no longer were recordable

DISCUSSION

In this study the factors affecting the conduction of activity were found to be primarily physical ones; most drugs tested having no effect upon the shape of peristaltic action potentials. Histamine lowered the threshold of conduction in ureteral smooth muscle but did not change the shape of the action potential. To date no means of restoring conductivity across ureteral anastomotic scars has been found. Antihistaminic drugs such as Benadryl caused a temporary disappearance of ureteral action potentials. These findings suggest that antihistaminic drugs may prove useful in the control of ureteral pain. Temporary relief of pain has followed the administration of Benadryl in 3 or 4 individuals suffering ureteral colic. The effects of histamine and antihistaminic drugs upon ureteral peristalsis suggest that histamine or some histamine like substance may be released by a contracting cell and activate contraction in neighboring cells.

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THE RENAL LYMPHATICS I THE EFFECT OF DIURESIS AND ACUTE URETERAL OBSTRUCTION UPON THE RATE OF FLOW AND COMPOSITION OF THORACIC DUCT LYMPH*

M K MYINT AND JOHN J MURPHY

Dissection of injected specimens in the dog and in man demonstrate that the major lymphatic channels draining the renal parenchyma make up 6 to 8 trunks surrounding the renal vessels (Fig 1). These trunks empty into the cisterna chyli or thoracic duct usually through 1 or more para-aortic lymph nodes. Lymph in the cisterna or thoracic duct then should reflect alterations in renal lymph, all other conditions being equal.

It has been suggested that the renal lymphatics might act as a route of decompression of the renal collecting system when the ureters are obstructed.¹ Direct communication between the renal pelvis and lymph channels have also been postulated. In an attempt to evaluate these theories and to elucidate the function of the lymphatics in normal and pathologic situations the following experiments were done:

*From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia, Pa.

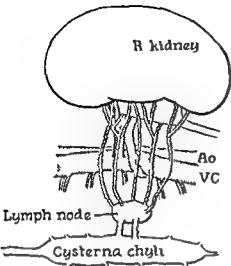


Fig 1 Diagram of anatomy of renal lymphatics

METHOD

Adult mongrel dogs were used as experimental animals and anesthetized with intravenous pentobarbital. The thoracic duct was exposed in the right chest and a polyethylene catheter was introduced into the cysterna chyli through the thoracic duct. The renal veins and the ureters were exposed transperitoneally. Baseline determination of rate of lymph flow and sodium chloride potassium urea nitrogen and total protein content of the lymph were made in all instances. Baseline values for these substances in renal and femoral vein blood were also obtained.

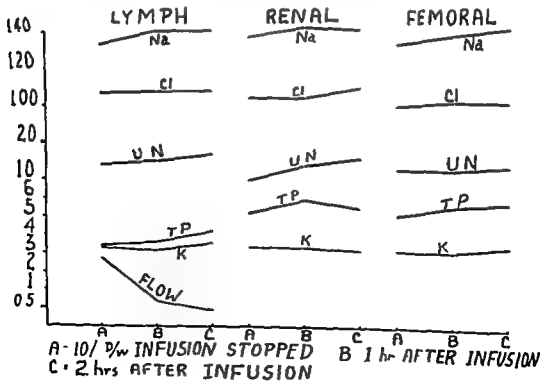


Fig 2 Effect of diuresis alone

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DISCUSSION

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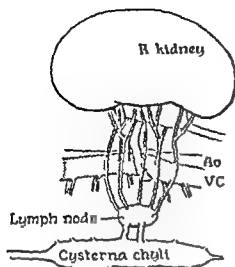


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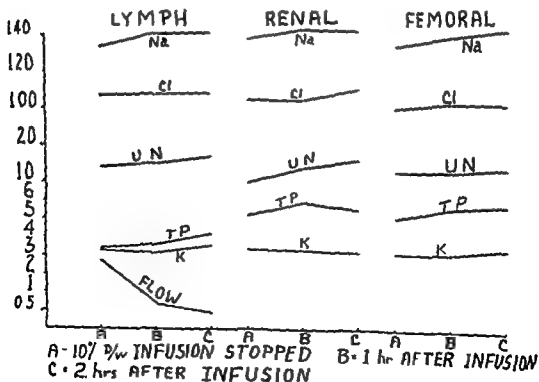


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DISCUSSION

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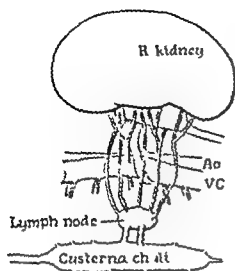


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METHOD

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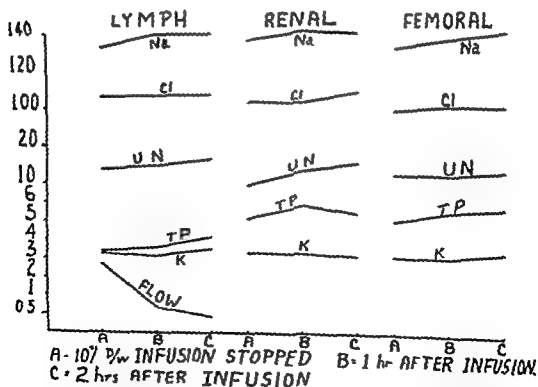


Fig 2 Effect of diuresis alone

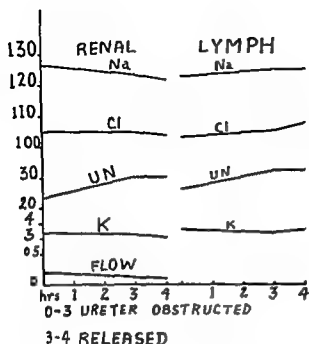


Fig 3 Effect of obstruction of 1 ureter in animal not in diuresis

DISCUSSION

Controls Animals in which the usual exposures and visceral manipulation were carried out showed only a transient rise in the rate of lymph flow. Values for urea nitrogen, total protein, sodium, potassium, and chloride were unchanged after visceral manipulation in the renal and peripheral vein blood as well as in the lymph.

Effect of Diuresis Diuresis induced by the rapid intravenous administration of from 300 cc to 500 cc of 10 per cent dextrose in water produced an increase in the rate of lymph flow in the cisterna chyli in each of 10 animals (Fig 2). Changes in the concentrations of sodium, potassium, chloride, urea nitrogen, and total protein were slight, tended to be similar in both blood and lymph, and seemed to reflect slight dilution of the intravascular fluids.

Effect of Ureteral Obstruction In 5 dogs obstruction of a single ureter with normal hydration (not in diuresis) resulted in no significant changes in the rate of lymph flow, on the sodium, potassium, and chloride content of the lymph. Urea nitrogen concentration rose slightly during the obstruction in both lymph and renal vein blood (Fig 3).

Obstruction of both ureters during diuresis in 6 dogs produced a significant increase in the rate of flow of lymph (Fig 4). The rate of lymph flow decreased when the diuresis ceased and the obstruction was released. Urea nitrogen in lymph, renal, and femoral vein blood increased during diuresis and obstruction and either leveled off or decreased slightly thereafter. Changes in sodium, chloride, potassium, and total protein paralleled each other in lymph and blood and again seemed to reflect the effect of intravascular dilution.

The changes observed in lymph flow and composition of lymph in diuresis confirms the impressions noted by Schmidt and Hayman¹ and supports the conclusions of Kohn² that renal lymph is derived from both renal plasma and tubular reabsorbed fluid. The failure of unilateral ureteral obstruction

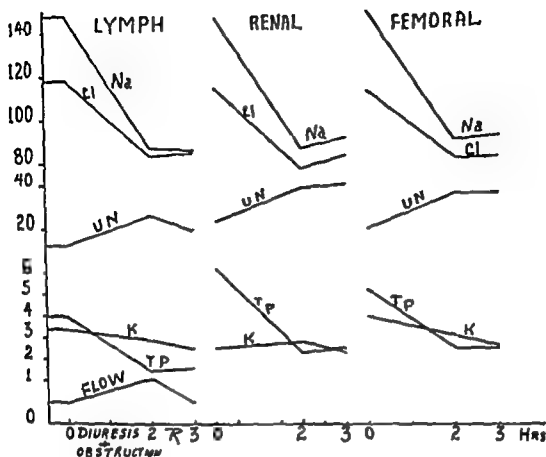
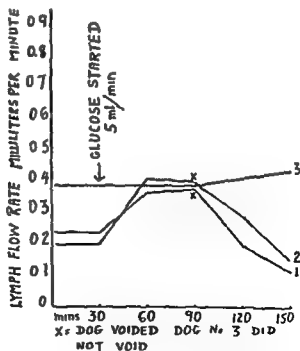


Fig 4 Effect of bilateral ureteral obstruction in presence of diuresis

Fig 5 Effect of voiding upon lymph flow



in a dog not in the state of diuresis to produce significant changes in rates of flow and composition of lymph suggest that the safety valve action of the lymphatics in ureteral obstruction is dependent upon definite pressure relationships between the lymphatic channels and the pelvis of the kidney

Thus the intrapelvic pressures which develop under the conditions described here for animals with unilateral ureteral obstruction not in diuresis are probably insufficient to cause pyelo lymphatic backflow whereas in those with diuresis and bilateral ureteral obstruction such pressures are developed rapidly and produce the phenomena described

An interesting observation was made during the course of these experiments with diuresis. In three animals whose ureters were intact, the bladders were seen to fill rapidly during diuresis. While the bladder was filling a rapid rise in the rate of lymph flow was noted in 2 of the animals (Fig 5). After 1 hr the rates of lymph flow remained unchanged for the next half hour whereupon 2 of the dogs voided with resultant rapid fall in the rate of lymph flow over the next hour. In the animal which did not void the rate of lymph flow slowly increased. The exact significance of this finding is not clear and further investigation is in progress.

SUMMARY

1 The rate of lymph flow in dogs is increased by diuresis induced by intravenous glucose in water

2 Obstruction of one ureter in a dog not in a state of diuresis produced no significant change in rate of flow and composition of the lymph

3 Obstruction of both ureters during diuresis produced increase in the rate of lymph formation and changes in the composition of lymph similar to those observed in renal and peripheral vein blood

4 The action of the renal lymphatics as a safety valve appears to be dependent upon definite pressure relationships between the lymphatic channels and the renal pelvis

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THE RENAL LYMPHATICS II. EFFECT OF INCREASING PRESSURE IN THE RENAL PELVIS UPON ABSORPTION OF SUBSTANCES OF VARIOUS MOLECULAR SIZES*

JOHN J. MURPHY AND M. K. MAYER

The fact that material in the renal pelvis may enter the circulatory system under certain conditions is well established. The routes by which transportation through the renal parenchyma is accomplished have been described as pyelo tubular, pyelo interstitial, pyelo venous and pyelo lymphatic. Evidence for each has been described by radiographic, hydrodynamic and radioactive tracer techniques. The exact role and relative importance of each route in normal and pathological situations is not clear. The experiments described here were designed to elucidate this problem.

METHOD

Adult mongrel dogs were anesthetized with pentobarbital given intravenously. The thoracic duct was exposed in the right chest, and through it a polyethylene catheter was passed into the cisterna chyli. The ureters were exposed transperitoneally and through them polyethylene catheters were passed to the renal pelvis and secured. A baseline rate of lymph flow was determined and control specimens of lymph, renal and femoral vein blood taken for analysis.

The renal pelvis was aspirated and then the pressure within the renal collecting system was gradually increased by adding increments of a solution of the test substance into it through a water manometer. Samples were taken of lymph and blood from the renal and femoral veins at intervals while the pressure was either kept constant or gradually increased. The samples were analyzed for the test substance being used and the results compared with the baseline levels. Graphs have been made with the concentrations of test substances plotted on the ordinate and pressure or time (if pressure was constant) on the abscissa.

RESULTS

In the first group of animals 50 per cent dextrose in water was introduced into the renal pelvis at 100 cm. of water pressure. The results are shown in Figure 1. The rate of lymph flow doubled in 10 min. then leveled off and slowly increased thereafter up to 70 min. Analysis of the lymph, renal and peripheral blood for glucose showed a marked increase of this material in the lymph in 10 min. and a constant increase thereafter. The renal vein blood showed a definite but slower rise and the peripheral blood slowly reflected these changes.

In another group of animals 50 per cent dextrose in water was introduced at gradually increasing pressures. The increments of pressure ranged from 10 to 20 cm. of water at 10 to 15 min. intervals. Figure 2 demonstrates the results obtained when samples of lymph and blood were taken at 15 to 20 min. intervals during this experiment and analyzed for glucose. It is apparent that the glucose concentration of the lymph began to rise sharply at

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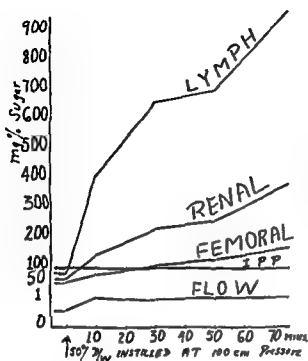


Fig. 1 Effect of 50 per cent D/W at 100 cm water pressure in renal pelvis upon rate of flow of lymph and concentration of glucose in lymph and blood from renal and femoral veins

30 cm of water pressure while in the renal vein such a rise was not seen until after 40 cm of water pressure was reached. The curve representing glucose concentration in the lymph tends to parallel that of the pressure while the renal vein blood concentration level off at 55 cm of water pressure.

In order to evaluate the transportation of a slightly larger molecule the dye T 1824 (molecular weight of about 980 as compared with glucose of

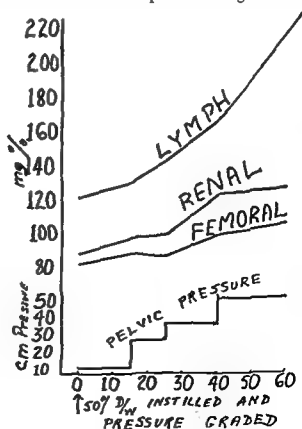
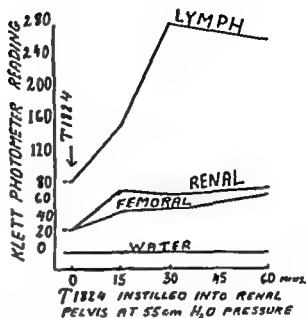


Fig. 2 Effect of 50 per cent D/W in stilling into the renal pelvis at gradually increasing pressure upon glucose concentration in lymph and renal and femoral vein blood

Fig 3 Photometric comparison of lymph and of plasma from the renal and femoral veins while the renal pelvis contain T 1824 at 5 cm H₂O pressure



180) was chosen. This substance can easily be identified visually and the concentration measured photometrically. Instillation of a solution of the dye into the renal pelvis at 5 cm of water pressure yielded some dramatic evidence regarding modes of transport from renal collecting system to the circulatory system.

Gross examination of the lymph obtained from animals during such an experiment clearly demonstrates the presence of the dye in increasing concentrations after 15 min. Visual comparison of samples from the cisterna renal and femoral veins up to 30 and 60 min leaves no doubt that the lymph contains much more of the dye than does the plasma.

A more exact comparison of dye concentration in such an experiment is shown in a graph. The Klett photometric reading of the lymph and plasma is plotted on the ordinate with time in minutes on the abscissa (Fig 3). Here again it is of interest that the concentration of T 1824 in the renal vein levels off after 15 min while that in the lymph continues to rise up to 1/2 hr.

Examination of a kidney sectioned after such an experiment demonstrates the dye beautifully outlining the renal pyramid and here and there extending through the cortex to the capsule. This is graphic evidence for the pyelo tubular and pyelo interstitial pathways. When combined with the evidence previously presented this makes a strong argument for the importance of the lymphatic route of transfer from the pelvis through the tubules and interstitial tissues to the circulatory system when increased intrapelvic pressures exist.

SUMMARY AND CONCLUSIONS

When introduced into the renal pelvis under pressure glucose and the dye T 1824 appear in lymph of the cisterna chyli earlier and in greater concentration than in the renal or femoral vein blood.

The appearance of the dye T 1824 in the renal tissues after its introduction into the pelvis in these experiments suggests that an important route of transfer under these conditions is from the pelvis through tubules and interstitial tissues and thence through the renal lymphatics into the circulatory system.

A STUDY OF URETEROSIGMOIDOSTOMY IN THE DOG WITH AND WITHOUT DIVERTING COLOSTOMY*

J. S. ANSELL AND C. D. CREEVY

The problem of renal insufficiency following urinary diversion is often attributed to ascending infection of the transplanted ureter. Infection may be the result of continual bacterial contamination and reflux from the bowel. In other instances scarring of the terminal ureter and of the intestinal wall through which it passes leads to stricture formation and secondarily infected hydronephrosis. Since Hinman's¹ masterful review of the subject new modes of urinary diversion have been described. Nesbit² advocated a wide spatulate end to side union between ureter and intestine with no attempt to prevent reflux. Leadbetter³ suggested a spatulate mucosa to mucosa anastomosis plus a sharply dissected submucosal trough for the terminal ureter. Goodwin⁴ fashions a similar trough by blunt dissection from within the bowel outwards. Ssolowoff⁵ suggests a tunnel for the terminal ureter made like that formed by Witzel around a gastrostomy tube. Mathison⁶ after studying avian and reptilian cloacae described a method of wrapping a full thickness flap of colon around the terminal ureter as it pierces the bowel to form an intraluminal nipple to prevent reflux. The use of diverting colostomy to prevent continued resoiling of transplanted ureters by the fecal stream has been suggested in clinical problems by the senior author⁷ and Vest⁸ recently and others in the past.

METHOD

This study surveys the above methods of ureterocolic anastomosis in the dog with and without diverting colostomy. Bilateral ureterosigmoidostomies were fashioned in 35 mongrel dogs of mixed sexes. Twenty of these dogs had diverting end colostomies. The proximal end of the distal colon was then closed and dropped back into the peritoneal cavity to provide a site for anastomosis and act as a reservoir for urine. In order to avoid influence of anatomical position on the results the various types of anastomoses were alternated from left to right side according to a predetermined plan. Dogs were fed low residue diets in the 48 hr. before operation and given 2.0 gm. Neomycin and 0.4 gm. Polymyxin B orally in 4 divided equally spaced doses. Penicillin 500,000 units and 0.5 gm. Streptomycin were injected intramuscularly twice daily during the first 4 days after operation. Distribution of anastomosis is shown in Table I.

One dog died with distemper on the first postoperative day. Another died of aspiration pneumonitis on the fourth day after operation. A third died of infarction of the proximal portion of the colonic reservoir down to and including the anastomotic sites. A fourth dog died on the thirty-second postoperative day with intussusception of the colonic reservoir. These 4 animals are included in the mortality for the series but not in the distribution table nor in subsequent statistics compiled on the basis of the length of time the urinary tract remained normal. A fifth dog died on the first postoperative day with a leak from a Goodwin anastomosis. The Mathison on the contra-

*From the Departments of Surgery, Divisions of Urology of the University of Minnesota Hospitals and the Minneapolis Veterans Administration Hospital.

evidence of pyelonephritis in the post mortem material examined. Tables 2 and 3 show some comparisons between Leadbetter, Goodwin, and Mathison ureterostomies. Table 4 shows the number of days urinary tracts remained grossly normal for all animals.

Table 3

DOG	COLO	DAYS GROSSLY NORMAL		FINAL CHEMISTRIES				
		GOODWIN	MATHISON	BUN	CO	Cl	Na	K
141	—	378	0	34	18	121	152	48
209	+	13	13	27	23	127	156	52
229	+	272	184	28	21	117	156	39
214	—	468	90	61	15	126	149	44
219	—	66	66	16	19	114	141	41
211	+	180	180	27	18	126	149	37
240	+	73	73	71	12	132	161	53
242	—	0						
Average		186	92	39	19	127	152	45

Table 4

LEADBITTER		DAYS GROSSLY NORMAL		NEFRIT		MATHISON			
		GOODWIN	WITZEL						
+	—	+	—	+	—	+	—		
0	281	33	378	60	62	47	60	162	
232	400	212	62	110	12	232	120	0	
404	225	468	66	213	44	46	53	12	
313	44	180	—		0	14	30	469	
800	587					42	513	33	
164	743						184	114	
0	60						164	90	
110	46						0	66	
62	463						180	73	
62	463						54		
							17		
A	248	331	324	160	141	39	81	118	113

Table 4. Days collecting system remained grossly normal as determined by Intravenous Urography or Post Mortem examination.

— with diverting colostomy

— without diverting colostomy

CONCLUSIONS

We conclude on the basis of these results that the difference between various techniques of ureterocolic anastomosis in the dog seems to be a quantitative rather than a qualitative one. When the ureter is transplanted to colon in the dog with or without diversion of the fecal stream ascending infection and renal failure eventually ensue.

The colon of the dog has no tenia, 3 muscular layers, and a plastic consistency that is quite different from the human colon. The anatomy of the canine colon may have had considerable influence on the results of this study.

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OBSERVATIONS ON THE OPERCULUM TECHNIQUE FOR URETEROENTEROSTOMY*

JACK LAPIDES JOHN M BOBBITT JAMES W MORROW AND
RUSSELL SCOTT JR

Although the majority of patients with bilateral ureterosigmoidostomy develop eventually pyelonephritis hydronephrosis and hyperchloremic acidosis a small number remain perfectly normal for periods as long as 20 yr or more Sigmoidoscopic examination of one of these normal patients revealed the ureteral orifices to be covered by overlying polypoid structures apparently arising from intestinal mucosa It was theorized that perhaps these mucosal flaps accounted for the normal upper urinary tract by preventing reflux of fecal material up the ureter and thus minimizing pyelonephritis In view of this consideration it was decided to develop a surgical technique whereby rectal mucosal flaps would be created to cover the ureteral orifice like an operculum

METHOD

The technique devised to create flaps of rectal mucosa is illustrated in Figure 1 The sigmoid colon is incised in a longitudinal manner along the antimesenteric border and a flap of mucosa is dissected free in the postero lateral region of the bowel lumen as shown in Figure 1 (1) The flap is about 1.5 cm long by 1 cm wide its free edges are approximated with fine catgut to form a tit (Fig 1 (2)) A small hemostat is then pushed directly through the bowel wall at the base of the flap the ureter grasped and drawn into the bowel lumen (Fig 1 (3)) The distal end of the ureter is spatulated and its free

*Section of Urology Department of Surgery University of Michigan Hospital Ann Arbor Mich

evidence of pyelonephritis in the post mortem material examined. Tables 2 and 3 show some comparisons between Leadbetter, Goodwin, and Mathison anastomoses. Table 1 shows the number of days urinary tracts remained grossly normal for all animals.

Table 3

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		GOODWIN	MATHISON	BUN	CO	Cl	Na	K
141	—	378	0	31	18	121	152	4.8
209	+	53	53	27	23	127	156	5.2
223	+	272	181	28	25	117	156	5.9
244	—	468	90	65	15	126	149	4.4
249	—	66	66	16	19	114	141	4.1
251	+	180	180	27	18	126	149	3.7
280	+	73	73	75	12	132	161	5.3
212	—	0						
Average		186	92	39	19	127	152	4.5

Table 4

LEADBETTER		DAYS GROSSLY NORMAL		WITZFL		NEBIT	MATHISON	
		GOODWIN						
+	—	+	—	+	—	+	+	—
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110	46						0	66
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62	464						54	
							17	
A	218 831	324 169		141 39		81	158 113	

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The colon of the dog has no more than 3 muscular layers and a plastic consistency that is quite different from the human colon. The anatomy of the canine colon may have had considerable influence on the results of this study.

visualization on one side. Autopsy revealed the absence of all the nipples except in one dog who had a short fibrotic tip. The ureteral orifices were patent in all animals with the exception of the dog demonstrating non visualization on one side. This animal had a complete stenosis of the ureteral opening. Although 11 of the 12 kidneys appeared to be grossly normal microscopic examination demonstrated chronic pyelonephritis to be present in all of them.

DISCUSSION

On the basis of the present experimental observations it would appear that mucosal flaps remain viable but disappear over a prolonged period of time due to retraction rather than atrophy. It is interesting to note that although the pyelograms appeared to be perfectly normal hyperchloremic acidosis was present associated with necropsy findings of chronic pyelonephritis.

SUMMARY

1. Intestinal mucosal flaps were constructed in dogs to act as flutter valves over the ends of the ureters. This was done in an attempt to prevent fecal reflux with resultant pyelonephritis and hyperchloremic acidosis.
2. The results of these studies reveal that the mucosal flaps remain viable for 2 to 4 wks but disappear after 6 mo. probably due to retraction.
3. Despite normal pyelograms hyperchloremic acidosis and chronic pyelonephritis were present in most of the dogs.

EXPERIMENTAL STUDY OF THE REGENERATION OF THE BLADDER IN DOGS*

M. H. THIAN, J. BRUNSON AND C. D. CREEVY

Although sporadic clinical observations have been made by Richardson¹ Folsom² and others that the bladder will regenerate after subtotal cystectomy extensive clinical application has not been made of this principle primarily because of the more popular techniques of diverting the urine into the intestinal tract after excision of the bladder. Experimentally the work of Berry³ Schiller⁴ and Kretschmer⁵ has shown that the bladder will regenerate in experimental animals after subtotal cystectomy. Bohne was the first to prove that the bladder will regenerate after total cystectomy. Little follow up has been available on clinical cases and the phenomenon of bladder regeneration has been observed in only a limited number of animals. The purpose of this experiment has been to study regeneration of the bladder more specifically both from a histologic and from a functional standpoint.

These experiments show that regeneration of the canine bladder does

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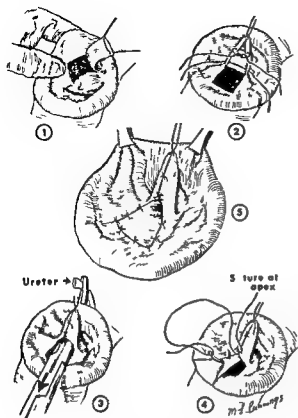


Fig 1 (1) Rectangular flap of mucosa dissected free (2) Approximation of edges of flap to form a tit (3) Grasping ureter to pull through bowel wall at base of operculum (4 5) An anastomosis of spatulated ureter to intestinal mucosa

edges sewn to the incised borders of the intestinal mucosa as well as to the base of the tit as demonstrated in Figures 1 (4) and 1 (5). The same method was utilized also to create flaps of bladder mucosa.

Initially dogs were autopsied 2 and 1 wks postoperatively to ascertain viability of the mucosal flaps. When it was established that the flaps remained viable, unilateral reimplantation of the ureter into the bladder of each of 3 female dogs was performed. Pyelograms and cystograms were obtained 3 mo after operation. The results of these urograms stimulated the performance of the operculum type ureterosigmoidostomy bilaterally in 10 dogs. All of these dogs were followed with interval blood chemistry studies and pyelography.

The animals with reimplantation of one ureter into the bladder were sacrificed at the end of 13 mo. Three dogs with bilateral ureterosigmoidostomy were sacrificed at the end of 6 mo and 3 more at the end of 11 mo. Autopsies were conducted on all the animals.

RESULTS

The urograms on the animals with unilateral reimplantation of the ureter into the bladder revealed normal pyelograms with no evidence of ureteral reflux. At autopsy examination of the bladder disclosed that the mucosal flaps had disappeared. Microscopic examination of the kidneys on the transplanted side revealed chronic pyelonephritis.

Blood chemistry studies on the dogs with bilateral ureterosigmoidostomy showed moderate hyperchloremic acidosis with normal blood urea nitrogen levels. Most of the pyelograms demonstrated normal collecting systems. However, one dog showed minimal hydronephrosis while another had non-

Three months after the initial operation blood chemistry and hemoglobin determinations were again made. If found normal an excretory urogram and cystogram was made and the capacity of the bladder was measured. Dogs were followed at 3 mo intervals thereafter with urigrams, cystograms, blood chemistry determinations and bladder capacity measurements. To observe the stages of regeneration histologically some dogs were sacrificed at intervals of 2 to 4 wks.

RESULTS

In all 12 animals were used. Table 1 shows the fate of all the animals. Two died within 24 hr following initial operation from leakage of urine into the peritoneum. One died 24 hr after the second operation due to anesthesia and 15 died of urinary obstruction and infection. Four of these also had distemper. All of these 15 dogs succumbed within 2 to 5 wks after excision of the bladder. At autopsy they had (1) pressure necrosis of the trigone from the prosthesis and (2) hydronephrosis and hydronephrosis from obstruction of the intramural ureter. Of the remaining animals 11 were sacrificed at intervals and will be discussed later. Ten are still under observation having now survived 6 to 12 mo following the initial operation.

As mentioned above 11 dogs were sacrificed at intervals after the initial operation, 2 each at the 2nd, 4th, 8th, 12th, 14th, 20th and 24th wks. The results of gross and microscopic study are as follows:

- (1) At 2 wks postoperative the prosthesis was surrounded by an inflammatory mass which upon microscopic examination consisted of acute inflammatory cells predominantly polymorphonuclear neutrophils.
 - (2) At 4 wks there was a rather thick walled inflammatory mass containing granulation tissue and many capillaries.
 - (3) At 8 wks there was globular mass with a smooth glistening inner surface. The outer surface was adherent anteriorly to the rectus sheath with some adhesions laterally and posteriorly to adjoining tissues. On microscopic examination the pouch was now lined by transitional epithelium.
 - (4) At 12 to 14 wks after operation the gross appearance differed little from the preceding but microscopic examination disclosed an inner layer of transitional epithelium with a base of granulation tissue surrounded by a zone of fibroblasts, lymphocytes and fibrous tissue intermingled with smooth muscle.
 - (5) At 20 to 24 wks after operation there was a pouch about 10 mm in thickness with smooth internal surface. Microscopically there was a multilayered inner zone of transitional epithelium and a middle zone of submucosal connective tissue. This was surrounded by a layer of smooth muscle which was thicker than before and which was covered externally by a layer of fatty tissue.
- Van Gieson's stain colored the smooth muscle yellow and the connective tissue deep crimson but did not reveal ganglion cells or other nerve tissue.
- Follow up. Intravenous urography was carried out on 28 dogs. Ninety per cent of the dogs revealed slight hydro-ureter at the first examination about 4 wks postoperatively presumably due to compression of the trigone by the prosthesis since it decreased after removal of the latter. Ten per cent had persistent hydronephrosis. Cystography in 16 dogs revealed a globular pouch. There was ureteral reflux in only one animal in which the ureter was inad-

occur that it is formed of smooth muscle lined by transitional epithelium and, although of limited capacity is capable of contracting at intervals and of emptying itself

METHOD

Adult mongrel female dogs weighing 15 kg or more were used. Nembutal 13 mg/kg body weight was used for intravenous anesthesia. Through a subumbilical median incision the bladder was exposed transperitoneally. It was dissected as widely as possible from the peritoneum which was closed with 00 chromic catgut to isolate the peritoneal cavity from the bladder area. The bladder was opened on its anterior aspect and the ureteral orifices identified. Number 4 French ureteral catheters were passed up the ureters and the bladder was resected leaving only the trigone. Bleeding around the cut edges of the trigone was controlled with 30 chromic catgut and the ureteral catheters were removed. A polyethylene ball with multiple 5 mm perforations was placed over the trigone. The prosthesis was covered with peritoneum and perivesical areolar tissue sutured to the cut edges of the trigone and internal urinary meatus. A stab was made over one of the holes of the prosthesis and a number 22 Malecot catheter was introduced through it into the lumen of the ball and led out through a separate stab wound in the abdominal wall. A nylon guard, to prevent the catheter from being bitten was threaded over the catheter which was anchored to the anterior rectus sheath with number 30 steel wire. The wound was closed in layers and the catheter was cut flush with the external opening of the nylon guard.

Four hundred thousand units of penicillin were given immediately after operation and twice daily for 3 days. The dogs were under observation for periods up to 12 mo. Four to 5 wks after operation the urea nitrogen, carbon dioxide combining power, serum chlorides, total serum proteins and hemoglobin were measured. If results were within normal limits an excretory urogram was made under anesthesia the following day. At the same time the polyethylene prosthesis was removed through a small incision. The bladder area was examined and irrigated thoroughly with normal saline and solution M* to wash out all the necrotic debris and soft sand. No attempt was made to divide the adhesions on its external surface. The pouch was then closed with interrupted 000 chromic catgut sutures. The rectus muscles and skin were approximated with No. 30 steel wire. Intensive antibiotic therapy consisted of 400,000 units of penicillin twice daily for 1 wk followed by 1 gm of gentrism orally per day for another week.

Table 1

No. of dogs operated upon	12
No. of dogs died 24 hr. 10	2
No. of dogs died 24 hr. after second operation	1
No. of dogs died from 2 to 5 wks	15
No. of dogs sacrificed	14
No. of dogs still alive	10
Total	12

Solution M: 1 lb. 4.5 Citric acid monohydrate 32.5 gm magnesium oxide anhydrous 3.84 gm sodium carbonate 8.84 gm distilled water 1000 cc

dog had a BUN of 60 mg which came down to 10 mg 3 days after removal of the ball and fluid therapy. The urea nitrogen was 20 mg 6 mo after the initial operation. Of the 15 dogs that died the urea nitrogen was measured in 5 and averaged over 60 mg per cent.

DISCUSSION

The difficulty of training the dogs not to bite the suprapubic tube has been solved by putting a nylon guard over the catheter. Urinary tract infection and stone formation has been a major problem because of the open suprapubic tube drainage. However if the bladder was washed thoroughly with saline and solution M at the secondary operation followed by an intensive course of antibiotics stones did not recur. The capacity of the bladder although not great did not decrease with prolonged observation. This study suggests that clinical application might be made of this principle in suitable cases. Such a method would be superior to urinary diversion for the following reasons: it is a less extensive operation, the intramural portion of the ureter is not disturbed, hence reflux is prevented and also the ureters do not come in contact with the intestinal contents. It is suggested that this method might be applicable in the treatment of extensive trauma of the urinary bladder following excision of localized carcinomas in exstrophy and following excision of the bladder for interstitial cystitis.

SUMMARY AND CONCLUSIONS

Regeneration of the canine urinary bladder has been studied following excision of the bladder save for the trigone and ureteral orifices. Early and late deaths occurred in 42.8 per cent of the dogs. Study of the remainder has shown that regeneration of a functioning bladder occurred in 75 per cent. Histological and functional studies of the regenerated bladder have been discussed.

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Fig 1 Photomicrograph of regenerated bladder of dog 12 wks after initial operation There is an inner layer of transitional epithelium below which is a zone of fibroblasts and smooth muscles

Fig 2 Cystogram of dog 6 mos following initial operation Note the globular smooth outline There is no reflux



Fig 3 Specimen of dog sacrificed 6 mo after initial operation The inner surface of the bladder is smooth and glistening This pouch was 10 mm in thickness Note the normal upper urinary tract

vertently severed during excision of the bladder The original capacity of the new bladder ranged from 30 to 40 cc In 10 dogs not sacrificed the bladder was stretched weekly by cautious over distension An average final capacity of 60 cc was obtained in 16 dogs with a range of 40 to 110 cc The animals did not dribble were continent and voided with a good stream

BUN CO₂ and chlorides were not much disturbed in 24 dogs Only one

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TOTAL MUCOSAL DENUATION OF THE CANINE BLADDER EXPERIMENTAL OBSERVATIONS AND CLINICAL IMPLICATIONS*

Preliminary Report

ARNOLD R. SANDERS CLARENCE J. SCHIFF AND LAZARUS A. ORSKIN

Recent studies in this laboratory have demonstrated the remarkable proliferative capacities of uroepithelium.^{1, 2} When autogenous arterial grafts were substituted for 10 cm segments of the canine ureter the graft endothelium was replaced by transitional cell epithelium within 6 wks. This epithelialization traversed the anastomoses from the intact ureter to line the entire graft. Since transitional cell epithelium did proliferate across defects of such length, a series of experiments was undertaken to determine whether it would reline the bladder of the dog whose mucosa had been completely removed and to evaluate the morphological and functional effects of such denudation upon the canine urinary tract.

METHOD

Male and female mongrel dogs ranging in weight from 17 to 35 lbs. were utilized without special preoperative medication. A standard veterinarian solution of nembutal was administered intravenously for anesthesia. The lower abdomen was opened through a left rectus muscle splitting incision and the urinary bladder was delivered onto the abdominal wall. The bladder dome was incised between Allis clamps and the incision was extended on the ventral surface down the neck.

A small wheal was raised in the mucosa at the superior edge of the opened bladder by the submucosal injection of saline which was then extended to cover the entire surface of the bladder by the further injection of saline. This converted the mucosa into a large bulla. A plane of cleavage was easily developed between the elevated mucosa and the muscularis and the mucosa was completely stripped from the underlying detrusor by blunt and sharp dissection. Hemostasis was obtained by the application of pressure to the denuded surface.

The bladder was closed around a #20 F Malecot tube with a continuous cutgut suture. Penrose drains were inserted into the prevesical space. The abdominal wall was closed in layers carefully extraperitonealizing the bladder stoma.

All animals were operated upon in this manner with one exception. In this dog a 2 mm cuff of mucosa was left intact around the left ureteral orifice.

Each dog received 1000 cc of 5 per cent glucose in water intravenously during the operation and 300,000 u of penicillin and 0.5 gm of streptomycin daily for 1 wk. after surgery. The tube and Penrose drains were removed on the seventh postoperative day. Serum sodium potassium CO₂ chlorides and blood urea nitrogen determinations were obtained on the

*From the Surgical Research Laboratory and the Urological and General Surgical Services, Montefiore Hospital, New York City. Aided by a grant from The Simon Holland Research Fund.

third seventh fourteenth and twenty first postoperative days. Intravenous urograms were taken postoperatively at 2 wk intervals for the first month after surgery and at monthly intervals thereafter. Retrograde cystograms were obtained at monthly intervals after the cystostomy sinus was completely closed and dry.

A total of 8 dogs was utilized for these studies. Two animals died: 1 on the third postoperative day from bronchiopneumonia and severe gastroenteritis. The second dog expired on the fourth postoperative day due to a technical error. In this case the cystostomy site had not been adequately extraperitonealized and urinary leakage eventuated in a generalized peritonitis. The remaining animals tolerated bladder denudation extremely well. They ate and drank with zest the morning following operation and showed no ill effects from the procedure. Urinary drainage was free of gross blood within 72 hr and the cystostomy sinuses closed within 10 to 12 days after removal of the tube. The animals apparently encountered no difficulty in voiding thereafter and by the fifth week after surgery their urines were clear and free of infection. Four dogs have been sacrificed to obtain anatomic and histologic data. The remaining 2 alive and well at present are being followed to evaluate the long term results of the procedure.

Blood Chemistries. A mild temporary elevation in blood urea nitrogen was noted in all animals for the first week after operation but this returned to normal by the fourteenth postoperative day. Other blood chemistries remained unchanged from preoperative levels.

Intravenous Urography. In 2 dogs the upper urinary tract was radiographically normal at all times. Initial postoperative excretory urograms in the remaining animals disclosed the presence of varying degrees of upper urinary tract dilatation ranging from a mild ureterectasia to a moderate hydroureter and hydronephrosis. However, this dilatation was transient and in all cases reverted to a normal radiographic pattern (Fig. 1).



Fig. 1A Excretory urogram. Left side shows mild pyelectasia and decreased lower tract capacity. Both lower tracts improved after operation. Ureteral dilatation and lower tract dilatation markedly decreased 60 days after operation. Note improvement in lower tract dilatation.

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Fig 3 Photomicrograph of re-epithelialized bladder 248 days after operation. Section taken from retrotrigonal area (hematoxylin and eosin, original $\times 200$)



dense connective tissue fibrils, blood vessels and a round cell infiltrate (Fig 3). Sections obtained from the dome and lateral walls of the bladder revealed a thinner epithelium with a more edematous submucosa, proliferating fibroblasts, numerous small blood vessels and fewer round cells.

The thickening of the vesical wall on gross examination seemed attributable to the changes noted in the submucosa. The muscularis and adventitia were not remarkable.

DISCUSSION

Total vesical denudation is a simple technical procedure which is well tolerated by the dog. A new uroepithelium derived from the ureteral and urethral stumps relines the bladder within 16 wks. There is no significant sacrifice of capacity. Postoperative upper urinary tract dilatation and reflux appear to be transitory and can be avoided entirely by retaining a small cuff of vesical mucosa around the ureteral orifices. These complications in the dog have been observed in this laboratory after other types of vesical surgery involving the trigone and may represent a species peculiarity.

That transitional cell epithelium would reinvest the totally denuded bladder is not surprising. Numerous investigators have demonstrated the remarkable proliferative capacities of uroepithelium in relining grafts used as ureteral and vesical substitutes. Bohn, Osborne and Hettle replaced the entire canine bladder with an acrylic mold.² A pouch-like substitute bladder was formed within 4 mo, completely lined by a uroepithelium derived from the intact ureters and urethra.

Clinically, total mucosal denudation might be applicable to the human with recurrent multiple papillomata of the bladder or multiple papillomatosis. Present day treatment of these growths is disappointing. Recurrence with eventual malignant degeneration and extension takes place all too frequently. Whether such tumors recur because of contact with the parent growth, arise independently from different foci within a tumor-prone bladder, or are the result of repeated exposures to known or unknown carcinogens is still controversial. However, their malignant potential is universally recognized and short of partial or total cystectomy, their long-term management has not been too successful.

Draper, Stark and Lau have suggested the replacement of the mucosa

Retrograde Cystography Initial retrograde cystography, performed on the 60th postoperative day disclosed the presence of ureteral reflux in all but one animal. The single exception was the ureter about whose orifice a 2 mm rim of mucosa had been left intact. Ureteral reflux was never demonstrable in this case. In 2 dogs subsequent periodic retrograde cystograms revealed a complete disappearance of reflux. In the others reflux persisted but with a marked diminution of the upper urinary tract dilatation noted on earlier studies.

Initial retrograde cystograms also demonstrated a moderate loss in bladder capacity estimated at 3 to 4 oz. However periodic retrograde cystograms thereafter showed progressive improvement and on the final study vesical capacity ranged from 8 to 10 oz. No evidence of bladder contracture was encountered in any animal.

Post Mortem Findings Examination of the 2 dogs which expired in the first week after operation disclosed mild dilatation of the ureters but normal kidneys. The bladder was congested and edematous in each animal. Its luminal surface was markedly irregular and covered with a gray exudate.

Dogs were sacrificed at 112, 120, 232 and 268 days after operation. In all instances the ureters and kidneys appeared grossly normal. No ureteral nor pelvic calyceal dilatation existed anatomically. Although the vesical walls revealed mild to moderate thickening the bladders were not contracted. In each animal the luminal surface was pink, smooth, glistening and appeared to be completely reepithelialized (Fig. 2). No encrustations nor calculi were found.

Histologic Specimens Tissue sections demonstrated that the urinary bladder was completely reepithelialized within 16 wks. The new mucosa appeared normal in respects and varied from 3 to 12 or more cell layers in thickness. Sections taken close to the ureteral orifices or urethra showed a greater depth of uroepithelium with a compact submucosa consisting of

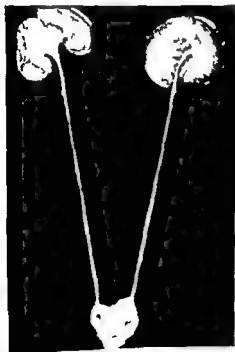


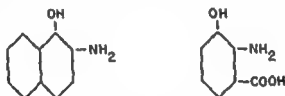
Fig. 2. Gross specimen 238 days after operation. Surface of bladder smooth, glistening and reepithelialized.

INVESTIGATIONS OF POSSIBLE ENDOGENOUS CARCINOGENS*

JEFFREY G. WATSON

This is a presentation of one facet of an investigatory program that is being carried out to determine the relationship of carcinogens and bladder cancer. The origin of this work began at the Chester Beatty Research Institute, London, England, and one phase of it was continued in the Section of Urology of the Department of Surgery, University of Michigan.

We investigated the possible carcinogenic properties of breakdown products of the amino acid tryptophan because we think that such products in the urine of man may be a cause of bladder cancer. These products, especially 3-hydroxyanthranilic acid, have a similar chemical structure to the known industrial carcinogen 2-amino-1-naphthol, the urinary carcinogen of 2-naphthylamine. Bonser¹ has shown that the conjugated form of



2-AMINO-1-NAPHTHOL : 3-HYDROXYANTHRANILIC ACID

2-amino-1-naphthol is not carcinogenic while its free or unconjugated form is carcinogenic. McDonald² demonstrated that the carcinogen 2-amino-1-naphthol must have actual contact with the bladder epithelium to produce the cancer. Boyland^{3, 4} found that in the urine of patients with cancer of the bladder there is an increase of both the enzyme glucuronidase and of the deconjugated breakdown substances that it produces from tryptophan metabolism. This was so even after the tumor had been removed. Boyland⁵ also showed that when given alone a certain carcinogen produced cancer in animals at sites other than the bladder but when tryptophan was given with this carcinogen then most of the animals developed carcinoma of the bladder. Thus it was thought that in some people an enzyme in the kidney or urine acts on a safe or conjugated product of tryptophan to produce a deconjugated or free amino phenol which given sufficient time to act on the bladder mucosa would produce bladder cancer.

METHOD

The endogenous or urinary breakdown products which we used were synthesized by Professor Boyland and were 3-hydroxyanthranilic acid, 3-hydroxykynurenine, 3-hydroxyacetophenone, xanthurenic acid, and kynurenic acid. A 20 per cent mixture of these substances was made up into 10 mg pellets using cholesterol as a base. 10 mg pellets of 100 per cent cholesterol were used as a negative control and similar pellets of a 20 per cent mixture of 2-amino-1-naphthol and cholesterol were used as a positive control.

These pellets were then placed into the bladder of mice and left there for 32 wks.

*Section of Urology, Department of Surgery, University of Michigan Medical School, Ann Arbor, Mich., and from The Institute of Cancer Research, Marsden Hospital, London.

of papillomatous bladders with skin grafts.¹⁻⁵ They removed the mucosa of the normal canine bladder and resurfaced it with thick split grafts of skin. Although the grafts were successful as evidenced by continued hair growth and corroborated by histologic section, pyuria, encrustations, calculi and bladder contracture developed. These complications have not been encountered in this series of experiments.

The results of canine vesical denudation encourage its possible application to the human with multiple recurrent papillomata or multiple papillomatosis of the urinary bladder. The procedure will aim at the extirpation of all papillomata together with the entire tumor prone mucosa. Whether upper urinary tract dilatation and reflux will occur in the human remains to be seen. However, in the dog these complications are not serious and tend to disappear. They can be prevented by leaving a small rim of mucosa around the ureteral orifices, but this defeats the purpose of the operation, namely, the removal of all vesical epithelium with its tumor potential. It is hoped that newly proliferated uroepithelium derived from the urethra and ureters, where the incidence of papillary growths is statistically low, will prove more resistant to neoplasia.

SUMMARY

- 1 A technique of total mucosal denudation of the canine bladder has been described.
- 2 The procedure is well tolerated by the dog.
- 3 The urinary bladder is completely reepithelialized in 16 wks.
- 4 Upper urinary tract dilatation and reflux are transitory sequelae.
- 5 The results of this study suggest its application to the human with multiple papillomatosis or multiple recurrent papillomata of the bladder.

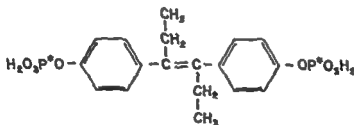
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DISTRIBUTION AND METABOLISM STUDIES OF P_3 LABELLED DIETHYLSTILBESTROL DIPHOSPHATE*

LESTER PERSKY, FREDERICK J. BONTI, AND JACK S. KROHMER

One of the aims of the chemotherapy of cancer is the differential localization of cytotoxic substances at the site of the neoplasia. A relatively new estrogen diethylstilbestrol diphosphate (stilbestrol) which has been used with moderate success in cases of far advanced carcinoma of the prostate¹ because of the nature of its chemical structure (Fig 1) suggested the possibility of achieving this therapeutic goal by the substitution of radioactive phosphorus (P_{32}) for the nonradioactive phosphorus in its molecule. The likelihood that the acid phosphatase of the prostate might selectively hydrolyze the drug had occurred to earlier workers^{2,3} who were hoping to depress prostatic cancer by the possible direct action of estrogen locally in high concentrations. The incorporation of a radioactive isotope into the molecule therefore seemed to offer a potentially double-barrelled therapeutic tool. The clinical observation that burning perineal pain rapidly attended the intravenous administration of diethylstilbestrol diphosphate offered some hope that this hydrolysis was occurring. Even though it has been noted that malignant prostatic tissue is relatively low in acid phosphatase⁴ it seemed worthwhile to do distribution and excretion studies in a series of experimental animals to test the validity of the hypothesis that we might successfully secure cancerocidal radiation doses in carcinoma of the prostate and its metastases. This same hypothesis had occurred independently to a group of British workers in a consideration of the effect of prostatic acid phosphatase on the drug *in vitro*.⁵



P_{32} LABELLED DIETHYLSTILBESTROL DIPHOSPHATE

Fig 1. Molecular structure of diethylstilbestrol diphosphate showing position of P_{32} .

METHOD

The dog was utilized as the experimental animal because of the higher levels of acid phosphatase present in its prostate⁶. Two series of animals were utilized. The control group consisted of dogs which received inorganic P_{32} as the phosphate ion intravenously. The experimental group received diethylstilbestrol diphosphate with substituted P_{32} . Because of the limited

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The mice, approximately 3 mo old and weighing 30 gm were anesthetized with an ether saturated cotton wool plug in a test tube then pinned onto a cork board and a transperitoneal cystotomy was performed using fine instruments

A small opening was made in the dome of the bladder the pellet inserted and the opening closed with a simple ligature The abdominal muscles and skin were brought together with a suture in each

After 32 wks the mice were sacrificed and autopsied In order to facilitate the histological interpretation of the bladder findings the bladders were distended with a 10 per cent solution of formaldehyde injected perurethrally They were then placed in 10 per cent solution of formaldehyde until fixed and then cut in the sagittal plane to produce a left and a right half of the bladder and each of these was examined histologically

RESULTS

Forty pellets of each of the substances were implanted Just over one half of the mice died during the experiment All the mice showed chronic cystitis but only the 3 hydroxyanthranilic acid ones showed epithelial changes Seven of the 19 survivors implanted with this substance showed marked epithelial changes consisting of several layers of cell nests as downgrowths of the epithelium These cells were atypical with a pale cytoplasm There was no infiltration In the same bladders there were also small papillary lesions containing epithelial downgrowths

Eight mice had a cast calculus of the bladder but none of these showed epithelial hyperplasia and these came from all groups

CONCLUSIONS

3 hydroxyanthranilic acid an unconjugated amino-phenol and a metabolite of tryptophan produced epithelial hyperplasia with downgrowths of cell nests while the other test substances and cholesterol produced only inflammatory changes Of the substances tested this one 3 hydroxyanthranilic acid most resembles the industrial carcinogens in chemical structure

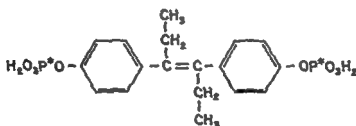
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DISTRIBUTION AND METABOLISM STUDIES OF P_{32} LABELLED DIETHYLSTILBESTROL DIPHOSPHATE*

LESTER PERSKY, FREDERICK J. BONTÉ AND JACK S. BROTHMER

One of the aims of the chemotherapy of cancer is the differential localization of cytotoxic substances at the site of the neoplasia. A relatively new estrogen diethylstilbestrol diphosphate (stilfostrol) which has been used with moderate success in cases of far advanced carcinoma of the prostate¹ because of the nature of its chemical structure (Fig. 1) suggested the possibility of achieving this therapeutic goal by the substitution of radioactive phosphorus (P_{32}) for the non radioactive phosphorus in its molecule. The likelihood that the acid phosphatase of the prostate might selectively hydrolyze the drug had occurred to earlier workers²⁻³ who were hoping to depress prostatic cancer by the possible direct action of estrogen locally in high concentrations. The incorporation of a radioactive isotope into the molecule therefore seemed to offer a potentially double barreled therapeutic tool. The clinical observation that burning perineal pain rapidly attended the intravenous administration of diethylstilbestrol diphosphate offered some hope that this hydrolysis was occurring. Even though it has been noted that malignant prostatic tissue is relatively low in acid phosphatase⁴ it seemed worthwhile to do distribution and excretion studies in a series of experimental animals to test the validity of the hypothesis that we might successfully secure cancerocidal radiation doses in carcinoma of the prostate and its metastases. This same hypothesis had occurred independently to a group of British workers in a consideration of the effect of prostatic acid phosphatase on the drug *in vitro*.⁵



P_{32} LABELLED DIETHYLSTILBESTROL DIPHOSPHATE

Fig. 1 Molecular structure of diethylstilbestrol diphosphate showing position of P_{32}

METHOD

The dog was utilized as the experimental animal because of the higher levels of acid phosphatase present in its prostate.⁶ Two series of animals were utilized. The control group consisted of dogs which received inorganic P_{32} as the phosphate ion intravenously. The experimental group received diethylstilbestrol diphosphate with substituted P_{32} . Because of the limited

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supply of the labelled drug in order to give adequate tracer doses only 5 animals were employed in each group. Two millicuries were used in each animal in both the control and experimental group.

After the intravenous injection of both materials samples of blood were drawn at 15 min, 30 min, 1 hr, 3 hr, 24 hr, 72 hr and after 1 wk. The dogs were sacrificed at $\frac{1}{2}$ hr, 1 hr, 3 hr, 72 hr and after 1 wk in each group. Specimens were taken of prostate, rib, muscle, liver, spleen and kidney in each animal. In dogs surviving for 24 hr or more, urines were collected daily.

All counts were made on dried or ashed samples using a thin end window Geiger counter. The counts were corrected for background and were compared to an aliquot of the original dose. The counts were then expressed as a percentage of initial dose per gram based upon wet sample weight.

RESULTS

Blood levels following injection of both labelled stilfostril and inorganic P_{32} are seen in Figure 2. The percentage of the initial dose is somewhat higher for the stilfostril than for the phosphorus alone. However, the slope of the curves are in general very similar. The results of counting of the tissues are represented in Table 1. In the interest of brevity and clarity, only the figures obtained after 1 day and after 1 wk are listed. In general, the earlier figures were very similar to the 24 hr percentages except in the case of the prostate and the liver in which higher percentages were found at $\frac{1}{2}$ hr, 1 hr and 3 hr.

In the case of the prostate using labelled stilfostril there was a definite increase in the percentage of the initial dose per gram found after 1 wk as compared to the 24 hr period. This was not true for the inorganic P_{32} which fell by approximately 50 per cent. After 1 wk the rib and spleen analysis remained the same as the first day analysis for the stilfostril but fell markedly for the P_{32} alone. The liver and kidney analysis fell approximately 50 per cent in both cases but the stilfostril started at levels twice as great in both instances. Muscle at the end of the week showed no deviation using inorganic P_{32} but rose by 50 per cent with the stilfostril P_{32} . The urinary concentration of the P_{32} in the stilfostril group was approxi-

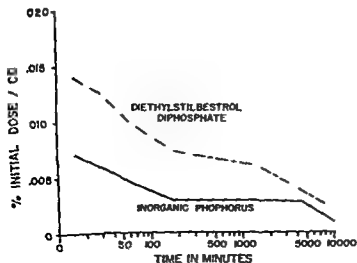


Fig 2 Disappearance curves of diethylstilbestrol diphosphate (P_{32}) and inorganic P_{32} from blood

Table 1 Percentage per gram of initial dose of diethylstilbestrol diphosphate labelled with P_{32} and inorganic P_{32} found on analysis of various tissues after one day and one week

	DAY		WEEK	
	SULFOSTROL	P_{32}	SULFOSTROL	P_{32}
Prostate	0.008	0.009	0.0128	0.001
Rib	0.009	0.01	0.009	0.005
Spleen	0.008	0.008	0.010	0.001
Liver	0.028	0.011	0.015	0.007
Kidney	0.026	0.014	0.014	0.006
Muscle	0.004	0.004	0.008	0.005

imately double that of the inorganic P_{32} and was approximately 1.5 per cent of the total dose per day following the first 24 hr.

DISCUSSION

The lower percentages of the initial dose found both early and late in our study is discouraging in terms of the therapeutic value of attaching radioactive phosphorus to the estrogen moiety of sulfoestrol. The rapid fall from the blood stream and the high concentration found early in the urine suggests that the phosphorus fraction can be expected to accomplish little in a cancerocidal sense. The diffuseness of its distribution similarly points to a failure of this aim. The counts found in the prostate after 1 wk suggest however that there is slight localization possibly due to the isotope still attached to the stilbestrol fraction. The failure to attain really high levels initially may be due to the rapid diffusion of the phosphorus into the large body pool or may be a function of the effect of acid phosphatase present throughout the body. The former impression is corroborated by the higher immediate counts found in the gland. In general however our studies confirm the impression of Marberger⁷ that there was little localization of this estrogen in prostatic tissue. We will not completely eliminate however any cancerocidal effect until the labelled compound has been assayed clinically since tumor may handle the drug in a different fashion.

The percentage of radioactivity still present in the rib after 1 wk is interesting but also discouragingly low. If the level of radioactivity had been higher we would have viewed with more optimism the administration of the drug to cases of widespread bony metastatic disease responding to estrogenic therapy. The failure to fall as did inorganic P_{32} may again be the result of that portion of the dose which remained unhydrolyzed. The initial high counts found in the liver are probably due in part to the liver's generalized role of detoxification and conjugation. The elevated renal counts at the start on the other hand are probably due to the renal clearance of the estrogen also still unhydrolyzed. The increased counts in the muscle after 1 wk further illustrates the diffuseness of the distribution.

Because of the small number of animals utilized and the limited amount of material available our figures and results cannot be analyzed statistically. It is possible that the acid phosphatase is not a specific enough enzyme for this type of treatment. It may be necessary to explore the efficacy of other isotopes in the place of the P_{32} which may too rapidly gain equi-

supply of the labelled drug in order to give adequate tracer doses, only 5 animals were employed in each group. Two millicuries were used in each animal in both the control and experimental group.

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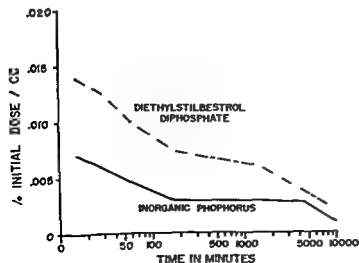


Fig 2 Disappearance curves of diethylstilbestrol diphosphate (P_{32}) and inorganic P_{32} from blood

in 1918 by a more extensive study.² All the reports of such examinations of New York Hospital patients between August 1915 and June 1931 have been reviewed. Follow up data for 5 yr are available in the cases of all but 1 of the 176 patients who had cancer cells found in their urine. Because our objective was to determine the reliability of the reports indicating cancer present rather than to evaluate the sensitivity of the test, no follow up was pursued in the other cases. The first 200 urine specimens studied from before August 1915 were not included as it was felt that during that early period the techniques and diagnostic criteria might be considered in the formative stages rather than based on extensive clinical applications.

During the period selected 3107 patients had 9216 reports of urinary sediment examinations or 2.93 studies per patient. On a few occasions several specimens gathered from a patient over a 1 or 2 day period resulted in a single report. The specimens were prepared and studied by the staff of the George A. Papirnicolou Cytology Laboratory in the Department of Anatomy. The specimens were classified as follows:

Class 0—No classification possible (too few cells or inadequate preparation)

Class 1—No evidence of malignant neoplasm found

Class 2—Atypical cells present but no evidence of a malignant neoplasm

Class 3—Cells present giving a suspicion of a malignant neoplasm

Class 4—Fairly conclusive evidence of a malignant neoplasm

Class 5—Conclusive evidence of a malignant neoplasm

In the first few years of these studies occasional specimens were reported in overlapping categories such as 3-4 or 4-5. When these were used for the purposes of this review the lower of the 2 categories was taken. Specimens were submitted by all departments of the hospital by whatever indications the individual physician wished to use. The series was not a cross section of the general population nor necessarily of a general urological or cancer practice.

Of the 9216 cytologic studies there were 112 (1.6 per cent) positive (Class 4 or 5). Of these 442 (39 per cent) were Class 4 and 267 (61 per cent) were Class 5. Of the 3107 patients who submitted urine for study 176 (5.7 per cent) had reports indicating the presence of cancer cells in the urine.

The sources of the exfoliated cancer cells in the urine are listed in Table 1.

The 11 unexplained reports of cancer cells in the urine concerned 8 patients. These 11 reports represent 2.1 per cent of all the positive reports in 9 patients or 4.6 per cent of the 176 patients with positive reports. It can be noted that the majority (72.7 per cent) of the unexplained reports were of Class 4. The follow up is practically absent in 1 case (NYH No. 477212).

There were numerous examples of patients with positive reports and without immediate urological evidence of cancer who on subsequent examinations have proven to have genitourinary cancer. The changes in circumstances from an unexplained report of cancer cells in the urine to proven genitourinary cancer has been so frequent with the passage of time that it is mandatory to follow indefinitely any such unexplained finding.

The positive smears that were unexplained in this group of old cases were recently reviewed independently by 2 staff cytologists. Perhaps the experience of an additional 5 or more years in evaluating these smears and improvements in criteria would affect the present day classification. All but

librium with the general body pool. The possibility also still exists that unattached radioactive isotopes may specifically localize in prostatic tissue as suggested by Gunn and Gould⁸ using Zn^{65} in the rat. Finally, since our current studies have not eliminated the possibility that the estrogen itself is organ specific we hope to continue this investigation with C^{14} labelled stilbestrols which will give us some clue as to the fate of the estrogenic portion itself.

SUMMARY

Preliminary experiments are described in which diethylstilbestrol diphosphate labelled with radioactive phosphorus was employed to determine the possible hydrolysis of this compound by prostatic acid phosphatase. These initial studies suggest only minimal localization of the isotope in the prostate as compared with other tissues. The slightly greater uptake, however, by prostate and bone suggests that further exploration is indicated with this and similar compounds and other isotopes.

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THE RELIABILITY OF THE PAPANICOLAOU TECHNIQUE WHEN CANCER CELLS ARE FOUND IN THE URINE*

SAMUEL I. ROLAND AND VICTOR F. MARSHALL

Early in 1945 cytologic studies of the urine were made available by the Papanicolaou Cytology Laboratory for the use of all departments of the New York Hospital-Cornell Medical Center. A preliminary report of these studies was made by Papanicolaou and Marshall¹ in 1945 and was followed

*From the Department of Surgery (Urology), Cornell University Medical College and the Department of Surgery (Urology), James Buchanan Brady Foundation of The New York Hospital. Aided by the staff of the Papanicolaou Cytology Laboratory and particularly by Dr. John F. Seybolt of the New York Hospital-Cornell Medical Center.

was done in this entire series of cases. The original classifications are the only ones used elsewhere in this review.

DISCUSSION

There is no doubt that occasional inexplicable or false positive reports of cancer cells in the urine will be made. Several such instances have been reported in recent medical literature.^{6, 7, 8} As in any laboratory aid to clinical evaluation this error, however small, must be kept in mind. The finding of a 1.6 per cent unexplained positive urine cytology in 3 of 175 patients with Class 1 or 5 urine reports has been sufficiently small to make us highly suspicious that a patient with such cells in the urine harbors a urinary cancer which may not have made itself evident in the usually recognizable forms. Five of these 3 patients were followed for over 5 yr. Studies of exfoliated cells in the urine have been a valuable part of the investigation of patients for urinary cancer, especially those cancers arising from the transitional epithelium. Cancers of the renal parenchyma and small prostatic cancers were not often detected by this method, but when cancer cells were found in such cases the report was reliable. Cytologic studies did occasionally push back the point of recognition of such disease and make possible its early diagnosis. It should be emphasized that these examinations were made by experienced cytologists.

SUMMARY

1. 9216 reports of the examination of urinary sediment smears from 3107 patients were reviewed.

2. 122.16 per cent of the 9216 reports indicated the presence of malignant neoplastic cells (Class 1 or 5).

3. These 442 reports were found in the cases of 176 patients, 5.7 per cent of the 3107 patients who contributed specimens.

4. Cancer has not been demonstrated in 8 (4.6 per cent) of these 176 patients. Five of the 8 patients have been followed for 5 or more yr. 2 have died without evidence of a urinary cancer prior to the elapse of 5 yr. and 1 patient has disappeared from follow up after 8 mo.

5. A number of impressive cases were found in which the Papancolaou method provided very early evidence of cancer—before careful urological examination did. Some of these cases are briefly described.

CONCLUSION

When it was reported that cancer cells were present in the urine the patient had cancer in the urinary tract in at least 95 per cent of the cases. A positive report which is unexplained warrants the following of the patient for an indefinite period of time.

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Table 1 Sources of the Exfoliated Cancer Cells in the Urine of 176 Patients

1 CASES PROVEN BY BIOPSY	NUMBER OF REPORTS AND CLASS		NUMBER OF PATIENTS
	4	5	
Renal carcinoma			
Pelvic	4	11	0
Parenchymal	2	1	2
Ureteral carcinoma	0	1	1
Bladder carcinoma	120	215	123
Bladder leiomyosarcoma	1	0	1
Bladder papilloma (Grade 1 carcinoma in some classifications)	7	7	6
Bladder papilloma with cystitis cystica	3	1	1
Prostatic carcinoma	22	14	10
Urethral carcinoma	2	5	2
Penile carcinoma	0	1	1
Penile melanosarcoma	0	1	1
Metastatic carcinoma to the urinary tract			
Adrenal to kidney	1	0	1
Stomach to kidney	0	1	1
Sigmoid to bladder	0	1	1
Ovaries to bladder	0	1	1
Rectum to bladder	3	0	2
Uterus to bladder	1	0	1
Pancreas with carcinomatosis	1	0	1
2 CASES UNEXPLAINED BY BIOPSY	8	3	8
Total	175	267	176

1 slide was available for review Table 2 is a review of the smears in the cases of inexplicably positive cytology of the urine

It can be seen that all but 2 patients had their smears re-classified so that cancer was not indicated with patients (NYH Nos 602036 and 136517) retaining almost the same classification. In only the last 2 cases did the two cytologists differ in each instance by only 1 class. It should be noted that the reclassifications cited in Table 2 are the only occasions a reclassification

Table 2 A Review of the Unexplained Class 4 and 5 Urine Papanicolaou Smear

NYH NO	DATE ORIGINALLY READ	ORIGINAL CLASSIFICATION	NEW CLASSIFICATION	
			CYTOLOGISTS	A B
436517	5 7 46	5		4 4
477212	6 2 47	4		3 3
221701	12 4 48	4		2 2
		4		3 3
		4		3 2
124492	3 6 49	4		2 2
565869	3 7 50	4		2 2
510953	4 7 50	4		2 2
591966	2 4 51	4		2 2
602036	6 5 51	5		5 4
	6 9 51	5		3 2

intrarenal vessels (the interlobular and arcuate arteries) consisting of narrowing irregularity and decreased number of vessels. These changes were consistent with chronic glomerulonephritis or arteriolonephrosclerosis. Eight other hypertensive patients who were known to have parenchymal renal disease were included in the investigation. The following diseases were represented: chronic pyelonephritis (3), bilateral hydronephrosis (1), congenital hypoplasia (1), radiation nephritis and Wilms tumor (1), traumatic pericapsular hematoma (1), and nephrocalcinosis (1). Three patients were considered to have aortograms inadequate for diagnostic purposes.

It is interesting to note that among the 53 nonhypertensive patients studied during the same period seven other patients were found to have nonobstructive renal artery disease. Four patients had congenital aneurysm, 1 had a large arteriovenous aneurysm, and 2 had calcified arteriosclerotic plaques within the wall of the renal artery without encroachment of the lumen.

Table 1 Pathology, grade of hypertensive disease, method and result of treatment of 16 patients with renal artery lesions found among 67 hypertensive patients studied by aortography, January, 1955, through August, 1956

PATHOLOGY	HYPERTENSIVE DISEASE	NUMBER OF PATIENTS	TREATMENT	RESULT
Arteriosclerotic plaques in main artery	Malignant	7	{ Nephrectomy Homograft Antipressor drugs	3 Cured 1 Dead postop hemorrhage 3 { 2 dead 1 unchanged
	Benign	3	{ Antipressor drugs	{ 2 unchanged 1 dead
Arteriosclerosis of branches	Malignant	1	Nephrectomy	Improved
	Severe	1	Attempted endarterectomy of solitary kidney	Dead—renal failure
Fibrous intimal proliferation	Severe	1	Bilateral arterial homograft	Cured
Thrombosis of main artery	Malignant	2	Nephrectomy	2 { 1 cured 1 improved
Stenosis with microaneurysms	Benign	1	Nephrectomy	Cured

Pathology. A considerable variety of renal artery lesions were found in the 16 hypertensive patients. Chief among these are thick obstructive arteriosclerotic plaques occurring in various locations along the renal artery. Ten patients were recognized as having this type of lesion and 3 of these demonstrated poststenotic or jet dilatation distal to the plaque.³ Arteriosclerosis involving the branches of the renal artery was found in 2 patients. One patient had fibrous intimal proliferation of both renal arteries.⁴ Two patients had thrombosis of the main renal artery with viability of part of the kidney assured because of an aberrant arterial circuit. The last patient had

- 1 Foot N Chandler and Papanicolaou G N Early renal carcinoma in situ J Am M Ass 139 356 358 1949
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- 11 Chute R and Williams D W Experiences with stained smears of cells exfoliated in urine in diagnosis of carcinoma of genito urinary system J Urol Balt 59 604 618 1918
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IRREVERSIBLE HYPERTENSION DUE TO RENAL ARTERY DISEASE*

E F POUTASSE, W J ENGEL, AND H P DUSTAN

Over 20 years ago Goldblatt showed that constriction of the renal artery of the dog produced hypertension. The clinical counterpart of this hypertension is now recognized but there are comparatively few well documented case reports.¹ From 1950 through 1954 we observed 6 patients who had either unilateral or bilateral obstructive lesions of the renal artery as the primary cause of hypertensive vascular disease. The 2 with bilateral lesions have been previously reported.² The 4 other patients had unilateral lesions and had reversal of their hypertensive state to normal by nephrectomy. All of these 6 patients had translumbar aortography with demonstration of the renal artery pathology.

Since 1955 aortography has been used on a much wider scale in the investigation of hypertension and the purpose of this paper is to present the results of this investigation.

Aortography was not employed routinely in the evaluation of patients with hypertension but was used in patients without family history of essential hypertension in the young (below the age of 35) without renal parenchymal disease in the elderly with accelerated hypertension and in those with a history suggesting the possibility of thrombosis or embolism of the renal artery.

Aortography in the Investigation of Hypertension From January 1955 through August 1956 109 patients were studied by aortography by the Urologic Service of these 67 were patients with all grades of hypertension from benign to the malignant hypertensive syndrome. Sixteen of the hypertensive patients (24 per cent) were found to have demonstrable obstructive lesions of the main renal artery or its immediate branches. 11 had unilateral and 2 had bilateral lesions. These obstructive lesions were considered to be instrumental in the onset and maintenance of the hypertensive state.

Eight others of the hypertensive patients had recognizable changes in the

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intrarenal vessels (the interlobular and arcuate arteries) consisting of narrowing irregularity and decreased number of vessels. These changes were consistent with chronic glomerulonephritis or arteriolonephrosclerosis. Eight other hypertensive patients who were known to have parenchymal renal disease were included in the investigation. The following diseases were represented: chronic pyelonephritis (3), bilateral hydronephrosis (1), congenital hypoplasia (1), radiation nephritis and Wilms' tumor (1), traumatic pericapsular hematoma (1), and nephrocalcinosis (1). Three patients were considered to have aortograms inadequate for diagnostic purposes.

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	Benign	3	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; font-size: 2em; margin-right: 5px;">{</div> <div style="display: inline-block; vertical-align: middle;"> Antihypertensive drugs </div> </div>	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; font-size: 2em; margin-right: 5px;">{</div> <div style="display: inline-block; vertical-align: middle;"> 2 unchanged 1 dead </div> </div>
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REVERSIBLE HYPERTENSION DUE TO RENAL ARTERY DISEASE*

E F POUTASSE W J ENGEL, AND H P DUSTAN

Over 20 years ago Goldblatt showed that constriction of the renal artery of the dog produced hypertension. The clinical counterpart of this hypertension is now recognized but there are comparatively few well documented case reports.¹ From 1950 through 1954 we observed 6 patients who had either unilateral or bilateral obstructive lesions of the renal artery as the primary cause of hypertensive vascular disease. The 2 with bilateral lesions have been previously reported.² The 4 other patients had unilateral lesions and had reversal of their hypertensive state to normal by nephrectomy. All of these 6 patients had translumbar aortography with demonstration of the renal artery pathology.

Since 1955 aortography has been used on a much wider scale in the investigation of hypertension and the purpose of this paper is to present the results of this investigation.

Aortography was not employed routinely in the evaluation of patients with hypertension but was used in patients without family history of essential hypertension in the young (below the age of 35) without renal parenchymal disease in the elderly with accelerated hypertension and in those with a history suggesting the possibility of thrombosis or embolism of the renal artery.

Aortography in the Investigation of Hypertension From January 1955 through August 1956 109 patients were studied by aortography by the Urologic Service of these 67 were patients with all grades of hypertension from benign to the malignant hypertensive syndrome. Sixteen of the hypertensive patients (24 per cent) were found to have demonstrable obstructive lesions of the main renal artery or its immediate branches. 14 had unilateral and 2 had bilateral lesions. These obstructive lesions were considered to be instrumental in the onset and maintenance of the hypertensive state.

Eight others of the hypertensive patients had recognizable changes in the

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and filling of the arteries themselves. The procedure is done under local anesthesia. The chief pitfalls in the diagnosis of renal artery lesions are the misinterpretation of an inadequate aortogram and the failure to appreciate the significance of even minor variations from normal in the renal artery or in the comparative sizes of the kidneys.

CONCLUSIONS

Obstructive lesions of the renal artery have been found to be associated with hypertensive vascular disease, presumably because of changes in the renal circulation. This hypertension may be benign or accompanied by a necrotizing vascular disease (malignant hypertension). Proper treatment, either by nephrectomy or arterial homograft, can cause remission of the hypertension.

Aortography is at present the most important means of diagnosis of renal artery disease.

Sixty-seven hypertensive patients were studied by aortography between January 1955 and August 1956. 16 of these patients were found to have renal artery obstructive lesions. This does not represent the true incidence of renal artery lesions in hypertensive patients. Eleven patients were treated surgically and, in 8, hypertensive disease cured or improved.

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partial stenosis with multiple congenital microaneurysms of the renal artery and 2 of its branches

Treatment Of the 16 patients with renal artery obstructive lesions 11 underwent surgical treatment. Seven had nephrectomy and thus far have had their hypertensive vascular disease either cured or improved. Of these 7, 6 had malignant hypertension. Four had normal blood pressure after nephrectomy. In 2 elderly patients the malignant syndrome disappeared and diastolic hypertension was controlled although systolic hypertension persisted. One patient underwent bilateral renal artery homografting to restore normal circulation to both kidneys with restoration of his blood pressure to normal.² One 30 yr old man with malignant hypertension and incomplete obstruction of one renal artery by 2 plaques died of hemorrhage 2 wks following attempted renal artery homograft. One hypertensive patient had excision of a congenital aneurysm with repair of the renal artery but the opposite renal artery has an arteriosclerotic plaque with distal jet dilatation. She is still under care at the present time. The patient with microaneurysms and stenosis of one renal artery has had normal blood pressure since nephrectomy. She had had asymptomatic hypertension for 3 yr. The eleventh patient had a solitary atrophic kidney and was found to be incurable by present surgical techniques because of arteriosclerosis with incomplete obstruction of all branches of the renal artery. He has since died of renal failure.

Five patients with renal artery obstructive lesions and hypertension did not undergo surgery. Two of these patients had far advanced malignant hypertension and died within a short time of progressive disease. They both had obstructing plaques at the orifice of one renal artery with a small kidney. One patient with a small arteriosclerotic plaque causing obstruction of one renal artery has failed to return for any treatment. One patient with bilateral obstructing lesions of the renal artery and severe hypertension died of widespread arteriosclerosis and massive cerebral thrombosis. The fifth patient is under antipressor drug therapy at present.

Diagnosis of Renal Artery Disease Intravenous urography should be performed in all non azotemic hypertensive patients. Unfortunately this study provides no consistent signs that would lead one to suspect the possibility of renal artery obstructive disease. There may be slight reduction in renal mass or slight reduction in function of the involved kidney but several patients with serious obstructive arterial lesions had what appeared to be a normal intravenous urogram. One patient with thrombosis of the main artery for example was considered to have essentially normal excretory renal function by urography probably because blood was still getting into the kidney through a large branch proximal to the thrombosis.

Comparison of the urine samples from each kidney in hypertensive patients is of considerable value. Kidneys with constricted arteries may show impairment of renal function as shown by the reduced concentration of substances in the urine as well as reduction in urine volume.

Translumbar aortography is the only means of demonstrating the presence of renal artery defects. The technique has been previously described.⁴ We have found that in patients with extremely elevated blood pressure it is helpful to use vasodepressor drugs to reduce the blood pressure and thereby obtain better compression of the aorta distal to the renal arteries.

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